This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

453
F5

$B 35 028

PHYSICAL CHEMISTRY

AND ITS APPLICATIONS IN MEDICAL AND BIOLOGICAL SCIENCE

2/- NET

FINDLAY

JUN 27 1906

LIBRARY
OF THE
UNIVERSITY OF CALIFORNIA.

Class

JUN 27 1906

LIBRARY
OF THE
UNIVERSITY OF CALIFORNIA.

Class

PHYSICAL CHEMISTRY

AND ITS APPLICATIONS IN MEDICAL AND BIOLOGICAL SCIENCE

BEING A COURSE OF SEVEN LECTURES DELIVERED IN THE UNIVERSITY OF BIRMINGHAM

BY

ALEX. FINDLAY, M.A., Ph.D., D.Sc.,

LECTURER ON PHYSICAL CHEMISTRY
UNIVERSITY OF BIRMINGHAM

LIBRARY
OF THE
UNIVERSITY
OF
CALIFORNIA

LONGMANS, GREEN, AND CO.
39 PATERNOSTER ROW, LONDON
NEW YORK AND BOMBAY
1905

[*All rights reserved*]

C24-3
F5

GENERAL

LIBRARY
OF THE
UNIVERSITY
OF
CALIFORNIA

PREFACE

The rapid development which has taken place in Physical Chemistry during the past two decades, and the success which has attended the application of physico-chemical principles and methods to the study of problems in Medical and Biological Science, render it of essential importance for students and workers in these departments to become familiar with the chief facts and theories, as well as methods, of Physical Chemistry.

As this Science is not usually included in the curriculum of study imposed on students of the biological sciences generally, it was with pleasure that the writer acceded to a request to give a short course of lectures on the subject, having in view, more especially, the requirements of members of the medical profession. No attempt at completeness has been made, for that would have been impossible in the short space of time available; but rather the desire was to give an elementary introduction to the subject, with the hope of encouraging and facilitating a more systematic and thorough study of physical chemistry on the part of students of medicine and biology.

In compiling these lectures the writer has received much assistance from the books by E. Cohen, *Vorträge für Ärzte über physikalische Chemie* (which has also appeared in English translation); R. Höber, *Physikalische Chemie der Zelle und Gewebe*; H. Koeppe, *Physikalische Chemie in der Medizin*; and especially from the standard work by H. J. Hamburger, *Osmotischer Druck und Ionenlehre in den medizinischen, Wissenschaften*; and he desires also to acknowledge the courtesy of the Editor in permitting the lectures to be reprinted from the pages of *The Birmingham Medical Review*.

A. F.

PHYSICAL CHEMISTRY AND ITS APPLICATIONS IN MEDICAL AND BIOLOGICAL SCIENCE.

By Alex. Findlay, M.A., Ph.D., D.Sc., *Lecturer on Physical Chemistry in the University of Birmingham.*

When we look back on the course of development of any of the branches of experimental science, we always observe that the progress of development is a discontinuous one, consisting of periods during which a rapid and vigorous development is succeeded by slower and feebler growth. Each of these generations in the life of the science begins with the conception of a new hypothesis and the birth of a theory which not only enables us to see the line of co-ordination binding together the isolated results of experimental enquiry, but also sends a beam of light, more or less intense, into the darkness of the unknown, thereby revealing paths along which further advance can be made. As advance proceeds farther and farther from the source, the light becomes fainter, and many facts and phenomena are encountered which are imperfectly illumined, and remain only partially understood until they also are lit up by the radiance of a new theory.

Thus it is that the great development in the study of solutions dates from the time when, in 1886, the Dutch chemist and physicist, van't Hoff, put forward his theory of solutions, and when, in the following year, the Swedish physicist, Arrhenius, propounded his theory of electrolytic dissociation of salts in solution. Not only did these twin theories co-ordinate a large mass of isolated facts and phenomena which were imperfectly understood, but they also opened up a new and large world for scientific investigation. Nor has the development which has taken place as the result of these theories been confined to physical chemistry; almost all the other experimental sciences have been markedly affected.

Since the laws of solution are nowhere of more importance than in connection with the animal and vegetable world, it is not surprising that the theories of van't Hoff and Arrhenius should have exercised an important influence on the development of medical and biological science. Thus we find that whereas, formerly, the function of biological chemistry was to investigate the nature of the compounds existing in plants and animals, and to discover the methods of isolating and producing them; recently, attention has been increasingly paid to the study of the vital processes taking place in the organisms, more especially in relation to the nature of the various solutions constituting the different body fluids of animals and the cell sap of plants. That work of great interest and importance has already been accomplished in this direction is known to every student of the biological sciences. One only requires to recall the work of Pfeffer and of de Vries on the application of the laws of osmotic pressure of solutions to the study of the movements and growth of plants; of Hamburger, on the hæmolysis of the red blood corpuscles; of Mascart and of Vladimiroff, on the relations between the osmotic pressure of solutions and their chemotactic influence on bacteria; as well as the similar experiments of Pfeffer on the spermatozoa of cryptogamic plants; and lastly, the almost startling experiments of Loeb on the influence of osmotic pressure and of certain ions in the production of artificial parthenogenesis.

In thus furthering the advance of biology, physical chemistry was only paying a debt which it owed to the biological sciences themselves; for it was largely owing to the experimental work of the botanist, Pfeffer, and of other biologists, that van't Hoff was able to establish his theory of solutions. While this is so, it can, however, also be said that the debt has been paid with interest, for by the introduction of the theories of solution, a development was assured to the biological sciences such as they probably would not otherwise have attained. That in this way the two branches of science—the physical and the biological—have been so mutually helpful, cannot but give the greatest satisfaction to all scientific workers.

In view of the great influence which physical chemistry is exercising on the development of the biological sciences, it was with great pleasure that I undertook, at the request of some of my colleagues, to give a short account of some of the more important theories and methods of physical chemistry, and to indicate some of the problems to the study of which these have been applied.

As it is probably the theories of solutions that have exercised the greatest influence on the biological sciences, it is with the properties of solutions that I propose chiefly to deal, and pass on in the first place to consider

DIFFUSION AND OSMOTIC PRESSURE.

When two gases are brought into contact, then, as you are aware, diffusion takes place—the one gas passes into the other—and this process continues until the whole gas mixture has become uniform in composition throughout. Thus, the light gas hydrogen will diffuse *downwards* into the heavy gas carbon dioxide, and the latter will diffuse *upwards* into the hydrogen. This process is rendered very evident by placing a small quantity of bromine at the bottom of a cylinder containing air. In a few minutes it will be seen that the bromine vapour, although more than five times as heavy as air, has passed up to the top of the cylinder. The driving force of this process is the difference of the partial pressures of the two gases at the different parts of the vessel.

A similar process also occurs in the case of substances in solution. If two solutions of different substances, or of the same substance but of different concentration, are brought into contact, diffusion also takes place, although much more slowly than in the case of gases; and this process also continues until the solutions become of uniform concentration throughout. This diffusion can be easily shown with a test-tube filled with a solution of gelatine to which a little phenolphthalein has been added, and placed mouth downwards in a dish containing a solution of caustic soda. The alkali diffuses up into the tube and reddens the phenolphthalein. I have chosen the experiment in this form because of the fact—a fact of importance in physiological work—that the addition of gelatine, agar-agar, and other substances of the same nature scarcely affects the velocity with which diffusion of salts and crystalloids takes place, although it prevents, or almost entirely prevents, the diffusion of other gelatinous or colloidal substances. The presence of the gelatine is also of value in that it prevents mixing by means of convection currents.

This diffusion of dissolved substances can also be shown by means of the familiar apparatus, the dialyser. If a solution of salt is poured into a vessel closed by means of parchment paper and placed in water, we find that after a short time some of the salt has passed through the parchment into the pure water, and this process will continue until the concentration of the salt is the same outside and inside the dialyser. In the case of gases the driving force we saw was the difference of the gas pressures; in the case of solutions the driving force is called the *osmotic pressure*.

How can this osmotic pressure be measured? If we place the dialyser with the salt solution in a vessel of pure water, we shall find that water will pass into the dialyser faster than solution passes out, so that the level of the solution will rise and produce a hydrostatic pressure. This is the phenomenon of endosmosis. The height to which the liquid rises, however, cannot be taken as a measure of the osmotic pressure of the solution, for the level of the liquid never remains stationary, but after reaching a certain height begins to fall again, until the levels outside and inside the vessel are the same. If we placed in the dialyser or endosmometer different solutions having the same osmotic pressure, we should find that the level to which the liquid rose was different in the different cases. The production of a pressure is to be ascribed to the difference in the velocity of diffusion of the dissolved substances through the membrane. The greater the velocity of diffusion, the lower will be the level to which the liquid inside the endosmometer will rise. We must therefore distinguish between the temporary pressure produced owing to the difference in the velocity of diffusion and the osmotic pressure. Both are of importance, and both may play a rôle in the processes of absorption and secretion.

Before we can measure the osmotic pressure of a solution, it will evidently be necessary to procure a membrane for the endosmometer which will allow the water to pass freely through, but will prevent the passage of the dissolved substance. Now, as you are all aware, it was found by Graham, many years ago, that parchment paper or animal membrane, such as pig's bladder, will allow water and dissolved salts to pass, but will prevent the passage of substances such as starch, albumen, and other so-called colloidal substances. Such a membrane, which is permeable to the solvent but not to the solute, or dissolved substance, is called *semi-permeable;* and we should therefore say that parchment paper is semi-permeable to a solution of albumen. If, then, we close the mouth of the endosmometer with parchment paper, and fill the vessel with a solution of albumen, the height to which the water will rise on immersing the endosmometer in water will measure the osmotic pressure of the albumen solution. The pressure in such a case is, for reasons which we shall learn presently, very small, amounting in the case of a 10 per cent. solution to only 0.17 atmospheres. Moreover, colloidal solutions are a special class of liquids differing in many respects from ordinary solutions, such as a solution of sugar or of salt; and it is not at all certain that the pressure which one really measures is the true osmotic pressure of the solution, in the same sense in which one uses this expression in the case of solutions of crystalloids.

However that may be, a parchment membrane cannot be employed for the determination of the osmotic pressure of solutions of crystalloids, because, as we have seen, these pass through the membrane. The first membranes semi-permeable for salts, were discovered in the thin membranes surrounding animal and vegetable cells, and the phenomena of osmosis were first studied by means of these. It was, however, at a later date, discovered by M. Traube that certain inorganic precipitates are semi-permeable with respect to the salts from which they are formed, and semi-permeable also with respect to many other dissolved crystalloids. Such a semi-permeable precipitate is, for example, copper ferrocyanide. When a solution of copper sulphate is mixed with a solution of potassium ferrocyanide, a brown precipitatate of copper ferrocyanide is produced, 2 $CuSO_4 + K_4Fe(CN)_6 = Cu_2Fe(CN)_6 + 2 K_2SO_4$, and this precipitate, while it will allow water to pass through, will prevent the passage of either copper sulphate or of potassium ferrocyanide, as well as of a number of other salts and crystalloids. Before such a precipitate could be employed for the investigation of osmosis, it had to be obtained in a coherent form as a membrane, and this Traube accomplished in the following manner. A strong solution of copper sulphate was allowed to run into a weak solution of potassium ferrocyanide. Immediately the copper sulphate came in contact with the solution of potassium ferrocyanide, a film of copper ferrocyanide was formed which enclosed the solution of copper sulphate, as it were, in a small pouch, depending from the surface of the potassium ferrocyanide solution.

Although Traube made a number of investigations of osmotic phenomena with the aid of these cells, it was not possible for him to actually determine the value of the osmotic pressure, because the thin membrane of copper ferrocyanide which was produced was, of course, too weak to sustain the high pressures which, as we shall presently learn, are obtained. The problem therefore arose as to how such membranes, in spite of their weakness, could be employed for the measurement of pressures amounting to several atmospheres. This problem was solved by the botanist, Prof. Pfeffer. Pfeffer took an ordinary battery pot of porous biscuit ware, and, after replacing the air contained in its pores by water, he filled it with a solution of copper sulphate and placed it in a solution of potassium ferrocyanide. Since the pot was, of course, permeable for both these salts, diffusion took place, the copper sulphate passing towards the outside of the pot, and the potassium ferrocyanide towards the inside. Somewhere within the walls of the pot the two salts would come together, and there form a precipitate of ferrocyanide. In this way a membrane was formed which was supported by the material of the pot, and which was thereby enabled to withstand considerable pressures. After being well washed, the pot was ready for use. A sketch of the apparatus is shown in Fig. 1. The porous pot, *z*, containing the solution the osmotic pressure of which was to be measured, was connected with a closed manometer, *m*, by means of the tube *t* and the collars *v* and *r*. The pot was then placed in pure water kept at constant temperature. In the course of a short time the pressure inside the pot is shown by the manometer to increase, and ultimately to become constant. The constant pressure thus produced represents the osmotic pressure of the solution.

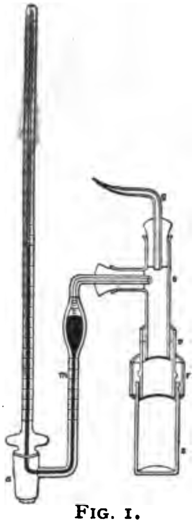


FIG. 1.

With this apparatus, Pfeffer carried out a number of determinations of osmotic pressure, and the pressures which he found were, even in the case of fairly dilute solutions, very considerable. Some of his results are given in the following table :—

TABLE 1.

SOLUTIONS OF CANE SUGAR. TEMPERATURE ABOUT 14°C.

Concentration by Weight.	Pressure.	$\frac{\text{Pressure.}}{\text{Concentration.}}$
1 per cent.	53.5 cm.	53.5
2 ,,	101.6 ,,	50.8
4 ,,	208.6 ,,	52.0
6 ,,	307.5 ,,	51.2

The numbers in the above table show that the osmotic pressure is directly proportional to the concentration. But the osmotic pressure also increases as the temperature is raised, as is shown in the following table giving the osmotic pressure of a 1 per cent. solution of cane sugar at different temperatures :—

TABLE 2.

Temperature.	Pressure.
14.2°	51.0 cm. Hg.
15.5°	52.1 ,,
32.0°	54.4 ,,
36.0°	56.7 ,,

Such is some of the experimental material accumulated by Pfeffer and utilised by van't Hoff in establishing his theory of solutions. According to this theory, *a substance in solution behaves as a gas occupying the same volume as the solution; and the laws which substances in solution obey are analogous to those which are followed by gases.* Let us just briefly recall what these gas laws are :—

(1) The first of these is that known as Boyle's law. This states that if the temperature is maintained constant, the pressure of a gas is inversely proportional to the volume occupied by it. If the volume is halved, the pressure is doubled; if the volume is doubled, the pressure is halved.

(2) The second law is that known as Dalton's or Gay-Lussac's law, which states that when the pressure on a gas is maintained constant, the volume increases as the temperature is raised, and in exactly the same way for all gases, viz., every gas expands by $\frac{1}{273}$ of the volume which it would possess at 0° for each degree rise of temperature. For the practical calculation of volumes, this law becomes simpler when stated in the form, that if the pressure is constant, the volume of a given amount of gas is directly proportional to the *absolute temperature*, that is, to the temperature measured from a point 273° below zero centigrade. Temperatures measured on this absolute scale, which we shall represent by T, will therefore be equal to the temperatures measured on the Centigrade scale plus 273°. If, now, we express the above law in symbols, we obtain $v : v' = T : T'$.

If, therefore, the volume is 1 litre at the temperature 273° A, *i.e.*, at 0°C., then, if the pressure is maintained constant, and the gas therefore allowed free expansion, the volume will be 2 litres when the absolute temperature is 546°, *i.e.*, at 273°C.

On the other hand, if the volume of the gas is maintained constant, the gas therefore not allowed to expand, then the pressure of the gas will increase in exactly the same way as does the volume when the pressure remains constant; in other words, the pressure which a given amount of gas exerts is proportional to the absolute temperature. That is: $p : p' = T : T'$.

(3) The third regularity which is observed in the case of gases is that summed up in the postulate of Avogadro. This very important postulate can be stated indirectly in the words: Under the same conditions of temperature and pressure, the gram-molecular weight of all gases occupies the same volume. By gram-molecular weight (also called a mole) is meant the molecular weight expressed in grams. Thus, the molecular weight of hydrogen is 2; a gram-molecule or mole of hydrogen is therefore 2 grams. In the case of nitrogen it is 28 gm.; of oxygen, 32 gm., and so on. If we choose the temperature of 0°C., and the pressure equal to one atmosphere, or 760 mm. of mercury, then it is found that the volume occupied by a gram-molecule of all gases is 22.4 litres. Therefore, 2 gm. of hydrogen, or 28 gm. of nitrogen, etc., at 0°C., and under the pressure of one atmosphere, occupy the volume of 22.4 lit. Knowing this, we can, of course, calculate, with the help of Boyle's law, what the volume would be under any other pressure; and with the help of Gay-Lussac's law, what the volume would be at any other temperature. Thus, if the pressure is 750 mm., and the temperature is 15°C., then the gram-molecular weight of any gas would occupy $\frac{22{\cdot}4 \times 760 \times 288}{750 \times 273} = 23{\cdot}95$ litres.

Further, since the volume occupied by a gram-molecule at 0° under atmospheric pressure is 22.4 lit., it follows that if we diminish the volume to one litre, the pressure will be 22.4 atmospheres. That is to say, *the pressure which is exerted by a gram-molecule of any gas when it occupies the volume of one litre at 0°C. will be 22.4 atmospheres.*

It is of essential importance to bear these laws in mind, because, according to the theory of van't Hoff, a substance in solution behaves as if it were a gas, and obeys analogous laws. In this case the volume of the solution corresponds with the volume of the gas, and the osmotic pressure of the solution with the gas pressure. This theory of van't Hoff we shall now proceed to test.

First of all, with regard to Boyle's law, that the pressure is inversely proportional to the volume. Let us again consider the values which Pfeffer obtained for the osmotic pressure of solutions of cane sugar (Table 1). With a 1 per cent. solution—that is, a solution containing 1 gm. dissolved in 100 gm. of water—the osmotic pressure was 53.5 cm. of mercury. If, now, we have the same amount of sugar in half the volume, *i.e.*, if we have a solution containing 1 gm. dissolved in 50 gm. of water (that is to say, a 2 per cent. solution), then if Boyle's law holds for dissolved substances the osmotic pressure ought to be 107 cm. mercury. Pfeffer found 101.6 cm. If we take a 4 per cent. solution, *i.e.*, if we make the volume in which 1 gm. of sugar is dissolved equal to one-fourth of the original volume, then the pressure ought to be 214 cm. Pfeffer found 208.6 cm. And so on. We see, then, that there is very fair agreement between the calculated values of the osmotic pressures, and those determined experimentally by Pfeffer. That the agreement is not better is to be ascribed to inaccuracies in the determinations, because other more accurate methods give results which confirm the law. (It may be mentioned that accurate measurements of the osmotic pressure by the method used by Pfeffer are exceedingly difficult; and further, the temperature in Pfeffer's experiments was not quite constant.) We can therefore say that Boyle's law for gases can be applied also to solutions, and state that, for a given amount of dissolved substance, the osmotic pressure is inversely proportional to the volume of solution. That is the same as saying that *the osmotic pressure is, for any given substance, directly proportional to the concentration of the solution.*

With regard, in the second place, to the effect of temperature. In the case of gases, the pressure of a given amount of gas is proportional to the absolute temperature. In the case of solutions, the analogous law would be: *The osmotic pressure of a solution of given concentration is proportional to the absolute temperature.* This law also is found to be true. Thus we saw (Table 2) that at 14.2° the osmotic pressure of a 1 per cent. solution of cane sugar is 51 cm. If the law just stated is true, then the osmotic pressure of the same solution at, say, 32°, should be (since 14.2° C = 287·2° A, and 32° C = 305° A), $\frac{51\cdot0 \times 305}{287\cdot2}$ = 53.4 cm. Pfeffer found 54.4 cm. Here again, therefore, we see that the osmotic pressure of solutions follows the same law as the gaseous pressure of a gas.

Lastly, with regard to the postulate of Avogadro. For gases we saw that at a temperature of 0°C., one gram-molecule of a gas exerts a pressure of 760 mm. of mercury when it occupies a volume of 22.4 lit., or 22,400 cc. The analogous law applied to solutions would be: At a temperature of 0°, the osmotic pressure of a solution would be 760 mm. (1 atmosphere), when it contains one mole of dissolved substance in 22,400 cc. of solution. We can test this as follows: One gram of cane sugar dissolved in 100 gm. of water gives a solution having the volume 100.6 cc. The molecular weight of cane sugar is 342. Therefore, 100.6 cc. of solution contain $\frac{1}{342}$ mole of cane sugar, and, therefore, 22,400 cc. will contain $\frac{22400 \times 1}{100\cdot6 \times 342}$ = 0.652 mole. The osmotic pressure of this solution at 0° was found by Pfeffer to be 49.3 cm.; therefore, according to our first law, the osmotic pressure of a solution containing one mole in 22,400 cc. would be $\frac{49\cdot3 \times 1}{0\cdot652}$ = 75.6 cm., which agrees very well with the theoretical value, 76 cm. Other substances give similar results, and hence we can state the very important law that at any given temperature all solutions which contain the same number of gram-molecules in the same volume have the same osmotic pressure; or, in other words, *equimolecular solutions of all substances are isosmotic.*

We shall see later that acids, bases, and salts are apparent exceptions to this rule; but we shall also learn how the behaviour of these substances can be brought into line with that of organic substances like cane sugar.

If the concentration of the solution is expressed not in gram-molecules per litre, but in grams per litre, then it follows that in solutions containing the same number of grams per litre, the osmotic pressure will be inversely proportional to the molecular weight. Thus, for example, if we have a substance of molecular weight 10, and another of molecular weight 40, and make solutions of each containing 10 gm. in a litre, the solution of the former substance will contain one mole, while the solution of the latter will contain only a quarter mole per litre. The osmotic pressure of the second solution will therefore be only one-fourth that of the first. The following table illustrates this :—

TABLE 3.

Substance.	Molecular weight.	Molar concentration* of a 10% solution.	Osmotic pressure in atmospheres.
Methyl alcohol ...	32	3.125	70.00
Urea	60	1.667	37.34
Grape sugar	180	0.555	12.43
Cane sugar	342	0.292	6.54
Albumen	13,000	0.008	0.17

(With regard to the osmotic pressure of the solution of albumen, it must be said that this cannot be taken as confirmation of what was stated above, because the molecular weight ascribed to albumen has been calculated from the osmotic pressure.)

It will further be observed from the above table that the osmotic pressure of a solution containing one mole in one litre will be equal to 22.4 atmospheres, which is analogous to what we found in the case of gases.

The importance of the law which has just been stated consists, of course, in the fact that if we know the molecular weight of a substance, we can at once calculate how much of it must be dissolved in a litre in order to give any desired osmotic pressure.

---

* That is, concentration expressed in moles per litre.

CRYOSCOPIC METHOD OF DETERMINING THE OSMOTIC PRESSURE.

Although, in discussing the laws of osmotic pressure, we have made use of the numbers obtained by direct measurement of the pressure in an osmotic cell, this method of determining the osmotic pressure of a solution is not a convenient one, and is never employed for ordinary purposes. The method which is almost exclusively used is the cryoscopic one, or the determination of the freezing point of the solution.

The principles on which this method is based are that when a solution begins to freeze, only pure ice, free from the dissolved substance, separates out; and that the temperature at which a solution freezes is lower than that at which pure water freezes. That is to say, the freezing point of water is depressed by solution in it of any substance, and the amount of the depression is proportional to the amount of dissolved substance.*

The importance of the cryoscopic method of determining osmotic pressures depends on the fact that solutions containing the same number of gram-molecules of dissolved substance in the same volume give the same depression of the freezing point. Thus, in the case of all substances (always excepting acids, bases, and salts), the depression of the freezing point of water when one gram-molecule of the dissolved substance is contained in 1 litre of the solution, is 1.85°C. Since the depression of the freezing point is proportional to the molar concentration of the solution, all solutions containing an equal number of gram-molecules in the same volume, freeze at the same temperature. In other words, *equimolecular solutions have the same freezing point.* But we have seen that equimolecular solutions are isosmotic; hence, solutions having the same freezing point are isosmotic.

It will be easy to see from this that determinations of the freezing point of solutions will enable us to calculate the osmotic pressure of the solution. Thus, we have seen that a solution containing one mole of dissolved substance in one litre causes a depression of the freezing point of 1.85°; and we have also learned that a solution of this concentration would have an osmotic pressure of 22.4 atmospheres. Since the osmotic pressure and the depression of the freezing point are each proportional to the molar concentration, we can calculate by simple proportion the osmotic pressure of a solution, the freezing point of which is known. Thus, the freezing point of human blood plasma has been found to be on an average, —0.56°. Since a depression of 1.85° corresponds with an osmotic pressure of 22.4 atmospheres, the osmotic pressure of blood plasma will be $\frac{22.4 \times 0.56}{1.85} = 6.77$ atmospheres.

---

* I omit here the description of the apparatus and of the special thermometer usually employed in carrying out this determination, as they can be properly understood only by actual acquaintance with the method in practice.

Emphasis may again be laid on the fact that it is not the percentage composition of the solution that is of value in giving the measure of the osmotic pressure or the depression of the freezing point, but the *molar concentration*, or the number of moles (gram-molecules) of dissolved substance in a given volume (1 litre) of the solution. Ordinary chemical analysis, therefore, which gives the percentage amount of dissolved substances, does not indicate the value of the osmotic pressure. For example, blood plasma contains about 9 per cent. of albuminoid substances, and about 0.8 per cent. of inorganic salts. But the freezing point of the plasma and of the serum, after removal of the albuminoids, is practically the same, so that the depression of the freezing point is due almost entirely to the inorganic salts. The reason of this is, of course, that the albuminoids have a very high molecular weight, so that a relatively very great amount of them by weight is required in order to produce even a comparatively small depression of the freezing point.

STUDY OF OSMOTIC PRESSURE IN PLANTS AND ANIMALS.

Turning from the consideration of the laws of osmotic pressure and the physical methods by which the osmotic pressure can be determined, we shall now consider some measurements of the osmotic pressure in the case of plants and animals. The first point which we shall take up is the osmotic pressure of cells, both vegetable and animal.

In investigating the osmotic pressure of the protoplasm of plant cells, the method most employed is the plasmolytic one, due chiefly to de Vries. In the case of many plant cells we find the cell surrounded by a sheath of cellulose, within which is the protoplasm surrounded by its membrane. When the cell dies, the protoplasm separates from the cellulose sheath, and the dissolved constituents of the cell sap pass out through the dead membrane. The same phenomenon—the separation of the protoplasm from its sheath—is observed when the living cell is placed in solutions the osmotic pressure of which is greater than a certain value. This is the phenomenon of plasmolysis. In the normal condition the protoplasm lies close to the cellulose wall, and maintains this in a certain state of tension. When the cells are brought into a strong salt solution, the osmotic pressure of which is greater than that of the cell sap, the cell contracts (owing to the passage outwards of water), and becomes flaccid. The protoplasm is here surrounded by a membrane which is semipermeable, allowing water to pass through, but not dissolved substances; or in any case it prevents the passage of many dissolved substances. By bringing cells into solutions of different concentration, we can find out the concentration at which plasmyolysis ceases. We then know that we are dealing with a solution which is isosmotic with the cell sap and the cell contents; and if we determine the osmotic pressure of this particular solution (by the croyscopic method, for example), we learn the osmotic pressure of the cell sap. Further, since solutions which are isosmotic (or isotonic) with the cell sap must also be isosmotic with one another, the method gives us a means of determining when solutions are isosmotic. But, on the other hand, having once determined the concentration of one solution which is isosmotic with the cell sap at a given temperature, we can calculate the concentration which the solution of another substance must have in order also to be isosmotic with the cell sap, provided we know the molecular weight of the substance in solution.

The turgidity or stiffness of plant stems, for example, is to be referred to the existence of the osmotic pressure in the cells. It will be increased by placing these in water or solutions of lower osmotic pressure than the cell sap (water will pass into the cells), and diminished by placing them in solutions of greater osmotic pressure (because water will now pass out from the cells).

Red blood corpuscles have also been employed in a similar manner for the determination of isosmotic solutions. When red blood corpuscles are placed in a strong salt solution, they shrink up and become crenated; when placed in pure water, they burst, and the red colouring matter is allowed to pass into the water. This again is due to the corpuscles being surrounded by a layer which acts as a semipermeable membrane. If the liquid in which they are placed has a lower osmotic pressure than the contents of the corpuscle, water will pass into the latter and cause the corpuscle to swell and finally burst. If the osmotic pressure is higher, water will pass out from the corpuscle, which will therefore shrink.

It must be borne in mind, however, that solutions which cause the bursting of the corpuscles are not really isosmotic with the contents of the latter. The membrane surrounding the corpuscles has a certain power of resistance, and therefore a certain difference of osmotic pressure will be necessary to cause its rupture. In order, therefore, to determine when a solution is really isosmotic with the corpuscle contents, we must ascertain the concentration which causes no change in the volume of the corpuscle. This is accomplished by means of the hæmatocrite. This consists of a small graduated capillary tube opening out at the top into a funnel-shaped expansion, to which a small syringe can be attached. A certain volume of blood is drawn up into the capillary, and after closing the two ends by means of caps, the tube is placed in a centrifuge. On being centrifugalised, the blood corpuscles collect at the end of the tube, forming a column of definite length which is read off. The hæmatocrite is then cleaned out, a fresh quantity of blood drawn up to the same mark as before, and then, following this, a quantity of the solution to be tested. The two liquids are mixed with a fine needle, and then centrifugalised as before. If the solution was hyposmotic, the length of tube occupied by the corpuscles will now be greater than in the first case; if the solution was hyperosmotic, it will be less. When the osmotic pressure of the solution is the same as that of the blood corpuscles, the volume occupied by the latter will be the same as when no solution was introduced.

Take the following example. With blood alone, the height of the column of corpuscles was, in four experiments, 52.5, 52.2, 52.5, 52.0; mean, 52.3. When mixed with solutions of cane sugar of concentration 6.84, 7.70, 8.55, 9.40 per cent., the readings were 56, 53, 51, and 49 respectively. The isosmotic solution, therefore, has a concentration between 7.70 and 8.55 per cent., viz., about 8.13 per cent., or contains about 0.24 gram-molecules per litre.

In applying this method, it must be borne in mind that it cannot be employed for determining the osmotic pressure of a solution when the dissolved substance can pass through the corpuscle wall. Thus, for example, urea can pass through the wall of red blood corpuscles, and addition of urea to a salt solution which is isotonic with the corpuscles causes no alteration in the volume of the blood corpuscles. The blood corpuscles are also permeable for alcohols, free acids, and alkalis, as well as for ammonium salts; but are impermeable for the sugars, such as cane sugar, milk sugar, and grape sugar.

Osmotic Pressure of Blood.—Since the most important body fluid of animals is blood, many experiments have been carried out on the osmotic pressure of this fluid. The osmotic pressure is most simply and easily determined by the cryoscopic method; and the method is rendered all the more simple on account of the fact that it makes practically no difference whether the experiments are carried out with blood as a whole—that is, including the corpuscles—or with blood plasma or serum. The corpuscles are suspensions, and do not affect the freezing point; and the presence of more or less albuminoid material is also of no practical importance, as we learned in our previous lecture.

On investigating the freezing point of the blood of different animals, it was found that the osmotic pressure of the blood is nearly the same in all cases. This is shown by the following table (Hamburger):—

TABLE 4.

Blood of	Freezing point.	Blood of	Freezing point.
Man ...	—0.56°	Dog ...	—0.599°
Horse ...	—0.580°	Rabbit...	—0.578°
Ox ...	−0.601°	Cat ...	—0.615°
Pig ...	−0.625°		

In all cases, therefore, with only slight variations, the freezing point of mammalian blood is about —0.6°, corresponding with an osmotic pressure of about 7 atmospheres (7.2)

Since the molar concentration of dissolved substance in the blood is subject to very great changes, owing to the introduction into it of varying quantities of dissolved salts and other substances, after partaking of food (and subject also to dilution after partaking of liquids), it might be asked, How does the osmotic pressure vary at different periods of the day? The following table gives particulars of two experiments on this point:—

TABLE 5.

	Freezing point of blood.		Freezing point of blood.
9 a.m.	—0.535°	9 a.m.	—0.512°
12 noon	—0.558°	11-15 a.m. ...	—0.551°
1-30 p.m. (after meal)	—0.585°	2 p.m. (after meal)	—0.617°
5-45 p.m. ...	—0.528°		

The osmotic pressure reaches a maximum shortly after partaking of the mid-day meal, due probably to the salt in the food. But although variations in the osmotic pressure do occur, these variations are, after all, only slight; so that the tissues are always surrounded by a liquid of almost constant osmotic pressure—a condition which is necessary for the healthy functioning of the cells and organs.

This regulation of the osmotic pressure of the blood is, of course, effected chiefly by the action of the kidneys, which excrete more or less concentrated urine according to the osmotic pressure of the blood. Hence, although the osmotic pressure of the blood remains nearly constant, that of the urine varies very greatly, say from 12 to 26 atmospheres, in twenty-four hours.

Since the regulation of the osmotic pressure of the blood is dependent on the healthy action of the kidneys, marked changes in the osmotic pressure of the blood will indicate impaired activity of the kidneys; and the determination of the osmotic pressure of the blood (by the cryoscopic method) has been proposed for the diagnosis of impaired function of one or both kidneys. Von Koranyi, who was the first to draw attention to this fact, showed that if only one kidney is affected, the osmotic pressure of the blood deviates but little from the normal; while, if both kidneys are impaired in function, the osmotic pressure may rise sometimes to 12.6 atmospheres, or the freezing point may change from —0.56° to —1.04°.

Another interesting observation which has been made with regard to the osmotic pressure of the blood is one which may be of considerable importance in medical jurisprudence. Although, in ordinary circumstances, no considerable difference between the osmotic pressure of arterial and venous blood is observed, it was found by Carrara that in cases of drowning the blood of the left heart shows a much smaller depression than that of the right. Thus, in the case of a dog. the blood from the carotid showed a freezing point of —0.60°, whereas, after drowning in fresh water, the corresponding blood from the left heart had the freezing point —0.29°, and that from the right heart —0.42°. If the animal was killed first, and then immersed in water for seventy-two hours, no marked difference in the freezing point of the blood was observed. One should therefore be able to say whether death was due to drowning or had taken place before the body came into the water.

It was further found by Carrara that if drowning occurred in sea-water, and not in fresh water, the relations were exactly the opposite; that is, the blood from the right heart has a smaller osmotic pressure than that from the left. In the case of a dog drowned in sea-water, the freezing point of the blood from the right half of the heart was —1.01°, and that from the left heart, —1.23°. This was confirmed in a case of accidental drowning of a man in sea-water. The blood from the right heart had the freezing point, —1.04°; that from the left, —1.18°. Such investigations of the osmotic pressure of the blood might be used for deciding whether drowning occurred in fresh or in sea-water. It must, however, be said that very few investigations appear to have been carried out in this direction; but the matter seems to be of sufficient importance to warrant its mention, and also its further investigation.

Before passing from the consideration of the osmotic pressure of the blood, mention may be made of the necessity of taking account of the osmotic pressure in preparing physiologically inactive solutions—physiological salt solution or saline. The physiological saline usually employed has a strength of about 0.7 per cent. This corresponds with an osmotic pressure of about four atmospheres. While this solution is isosmotic with the blood of the frog, and can therefore be used in preserving the tissues of that animal, it is hypo-osmotic to blood of mammals, and would therefore not be so suitable for preserving the organs of mammals, or for injection to replace loss of blood. The physiological saline for mammals should contain about 1 per cent. of salt (sodium chloride), corresponding with an osmotic pressure of about seven atmospheres.

With regard also to the fixing of animal structures, it does not follow that a solution which is isosmotic with the animal juices will cause no change in the volume of the structure, because of the permeability of the cell walls to certain substances. Thus, solutions containing formaldehyde, which are commonly used for fixing animal tissues, cause increase of volume. This is due in part to the presence of formic acid, to which the cell walls are permeable, and can be diminished by neutralisation. Even when that is done, however, a slight increase of volume may be observed, possibly on account of changes produced in the protoplasmic substance.

LIBRARY
OF THE
UNIVERSITY
OF
CALIFORNIA

Osmotic Pressure and Chemotaxis.—I should now like to say a few words with regard to the relation between osmotic pressure and chemotaxis, and the first case we shall take is that of the effect of osmotic pressure in the production of artificial parthenogenesis.

The first experiments in this direction were carried out by Loeb with the eggs of echinoderms. In ordinary sea-water, these eggs, if not fertilised, decay; but if the osmotic pressure of the sea-water is increased by 30 to 60 per cent., most, though not all, undergo parthenogenic development to a free swimming larva. In these experiments it seemed to be of no consequence what substances were employed in order to increase the osmotic pressure, and development would seem to be due to the stimulation caused by loss of water by the ovum.

While it was found that in the case of some of the echinoderms, especially sea-urchins, parthenogenic development occurred by raising the osmotic pressure of the medium, it was found that in other cases, *e.g.*, in the case of the chaetopterus, that when unfertilised ova are placed in sea-water to which a small quantity of a potassium salt has been added, the ova develop to apparently normal larvæ. Such a solution may have an osmotic pressure scarcely higher than that of sea-water, and has no effect on the eggs of the sea-urchin. Further, the effect in the case of chaetopterus can be produced only by potassium salts; other salts do not act. Similar parthenogenic development could be produced in the case of the ova of other animals, but it was found that different kinds require different salts. Thus, in the case of the star fish, parthenogenic development could not be produced by potassium salts, but could be effected by acids. In the case of amphitrite, development can be produced by adding calcium salts to sea-water; but the salts of magnesium, strontium, lithium, potassium, or sodium have no effect. We shall refer to this again later.

Interesting as these experiments are in the study of embryology, no satisfactory theory of the action of the salts has been given, nor have they so far given an interpretation of the natural process of fertilisation.

Other chemotactic influences of the osmotic pressure have also been found in the case of bacteria. The mobility of bacteria in salt solutions of varying concentration was studied especially by Mascart. A small quantity of bacteria culture was placed on a cover glass, which was then inverted so as to give a hanging drop, and placed under a microscope on a small frame of cardboard. Solutions of varying concentration were placed in capillary tubes, and a small quantity of potassium carbonate added to each. The ends of the capillary tubes were then brought into the drop of culture. When the capillary contained only potassium carbonate, the bacteria flocked into the capillary; but as the concentration of the other salts in solution was increased, the number of bacteria which entered diminished more and more; and if the concentration increased above a certain amount, the bacteria were even repelled. (Bacteria employed : spirillum undula, and bacillus megatherium.) On investigating the osmotic pressure of the solutions, Mascart found that equimolecular solutions (isosmotic) had all the same effect. Thus, solutions containing 0.004 mole of salt in 100 cc. of solution allowed the bacteria to enter; if the strength rose to 0.005—0.006 mole per 100 cc., the bacteria remained at the mouth of the tube, and if increased above this the bacteria were repelled. That is, a solution of sodium chloride containing 0.007 mole = 0.007 × 58.5 gm. = 0.41 per cent. of sodium chloride, repelled. The action depends, then, only on the osmotic pressure of the salt solution. Bacteria are, however, not all equally sensitive to changes in concentration, and they also possess a certain power of adaptation. If cultivated in a medium containing small quantities of a salt, they become less sensitive to that salt, so that larger concentrations are required in order to repel them.

One further point may be referred to before leaving the subject of osmotic pressure and its action, viz., the poisonous action of pure water. If tissues or cells are placed in distilled water, passage of water into the cells occurs owing to the difference of osmotic pressure. The cells swell up and may finally burst and die. In this way pure water will act as a protoplasmic poison. A similar poisonous action on cells is observed when distilled water is drunk. In this case the surface layers of the epithelium of the stomach undergo considerable swelling; salts also may pass out, and the cells die and be cast off. This may lead to catarrh of the stomach. It is to this action of pure water that the harmful effect of melted snow or ice is due, since freezing purifies the water. For this reason, also, one of the springs at Gastein has come to be known as the Poison spring, although its water is purer than ordinary distilled water.

THEORY OF IONISATION.

WHILE discussing the laws of osmotic pressure, it was stated that equimolecular solutions of different substances have the same osmotic pressure; and also, in speaking of the depression of the freezing point of water, that equimolecular solutions produce the same depression. These rules, however, do not apply to acids, bases, and salts.

Thus, for example, if we dissolve, say, 1 mole (58.5 gm.) of sodium chloride in water and make the volume of the solution up to a litre, the freezing point would be not —1.85°, but about —3.35°. Similarly, the osmotic pressure of such a solution would not be 22.4 atmospheres, but about 40 atm. A molar solution of sodium chloride, therefore, behaves as if it contained not 1 gram-molecule, but $\frac{3{\cdot}35}{1{\cdot}85} = 1.8$ gram-molecules of salt in a litre. In order, therefore, to find what the actual effect of such a solution would be as regards osmotic pressure, we must multiply the normal value of the osmotic pressure by a factor, greater than unity, in the present case, 1.8, in order to obtain the actual osmotic pressure. This factor is called the isotonic coefficient, or sometimes van't Hoff's factor, and is represented by i.

To illustrate this abnormal behaviour of electrolytes, I give one or two examples from the work of de Vries, who employed the plasmolytic method :—

TABLE 6.

Substance.	Molecular weight.	Plasmolytic concentration.	Molar concentration per litre.	Isotonic coefficient.
Cane Sugar	342	6%	0.175	——
Sodium Chloride...	58.5	0.65%	0.111	1.57
Potassium chloride	101	1.2%	0.119	1.47
Potassium sulphate	174	1.43%	0.082	2.12

Similar numbers also are obtained from measurements of the freezing point. Thus, a solution of sodium chloride containing 0.539 moles (=31.55 gm.) per litre, gave a freezing point of —1.85°, whereas a solution of cane sugar containing 0.539 moles per litre (=184 gm.) would give a freezing point of $-1.85 \times 0.539 = -0.997^\circ$. Hence, for the solution of sodium chloride, $i = \frac{1{\cdot}85}{0{\cdot}997} = 1.855$.

We can, therefore, state the general law that *salts, acids, and bases when dissolved in water give solutions possessing an abnormally high osmotic pressure and an abnormally low freezing point.*

Now, not only do salts behave abnormally, but the behaviour is all the more abnormal the more dilute the solution : *i.e.*, the isotonic coefficient increases with dilution. Thus, whereas the isotonic coefficient of a 3.15 per cent. solution of sodium chloride is 1.85, that of a 0.27 per cent. solution is 2. In other words, the last solution has twice as great an osmotic pressure as what one would calculate simply from the molecular weight of the salt.

Without delaying too long over this point, I give in the following table some values of the isotonic coefficient for 1 per cent. solutions of different substances :—

TABLE 7.

	i		i		i		i
KCl	1.82	K_2SO_4	2.11	KOH	1.91	HNO_3	1.94
NaCl	1.90	K_2CO_3	2.26	NaOH	1.96	H_2SO_4	2.06
KNO_3	1.67	Na_2CO_3	2.18	HCl	1.98	H_3PO_4	2.32

We see, then, that the abnormal behaviour of salts, acids, and bases, as compared with organic substances, consists in the fact that they behave as if they contained more molecules of dissolved substance than one calculates from the molecular weight of the substance. It looks, therefore, as if the molecules of salts, acids, and bases break up in solution so as to yield a larger number of molecules—each of the parts thus formed behaving as a separate molecule.

Now we meet with a precisely analogous phenomenon in the case of gaseous substances. Most of you will remember that when ammonium chloride is heated, it passes into vapour; but this vapour not only contains the molecules NH_4Cl, but also NH_3 and HCl, formed by the dissociation of the molecules of $NH_4 Cl$. And we should find that if we had 1 gram-molecule of ammonium chloride in an exhausted vessel of 1 litre capacity, and we caused the salt to pass into vapour, the pressure which would be produced would be greater than with a gram-molecule of hydrogen under the same conditions of temperature.

Since we see, then, that there are substances which in the gaseous state dissociate or break up so as to yield a larger number of molecules, it appears a not unnatural assumption to make that when salts, acids, and bases are dissolved in water, they also dissociate; because, as we saw, their solutions behave as if the number of dissolved molecules were greater than that calculated from the molecular weight of the dissolved substance. If, however, we make this assumption, then the question at once arises, what then are the products of dissociation? If we take sodium chloride as an example, we shall ask: Into what substances does this dissociate when the salt is dissolved in water? Clearly, not into sodium and chlorine, for sodium is a metal which decomposes water with evolution of hydrogen, and chlorine is a gas which dissolves in water, imparting to it a characteristic odour and bleaching properties.

To obtain an answer to our question, we shall consider some other properties of the abnormal solutions. Take, first of all, the case of acids. If an acid is dissolved in water, you know that the solution has the power of reddening blue litmus. Thus, no matter whether we take HCl, HNO_3, H_2SO_4, $H_2 T$ (tartaric acid), this reaction is given. Now, the only element which these substances have in common is hydrogen, and we should therefore be tempted to say that the reddening of litmus is due to hydrogen. But you know that hydrogen gas does not redden litmus, and also you know that there are many substances containing hydrogen which, even when dissolved in water, do not possess the property, *e.g.*, cane sugar, $C_{12}H_{22}O_{11}$. Further, we find that acids redden litmus only when in solution; dry tartaric acid and pure liquid hydrogen chloride do not possess the property. We must therefore conclude that the reddening of litmus is due not to hydrogen in the ordinary state, nor even to hydrogen in combination, but to a form of hydrogen which exists only in solution, *i.e.*, to some allotropic modification of hydrogen. Whenever that form of hydrogen is present in solution, we get the reddening of litmus; when it is absent, the solution does not give the reaction. For convenience, we shall designate this allotropic modification of hydrogen by $H^{\cdot}$.

What about the chlorine? You know that when silver nitrate is added to a solution of hydrochloric acid, a white precipitate of silver chloride is obtained. But this white precipitate is also given by NaCl, KCl, $BaCl_2$, etc. Now the only common element here is chlorine. But the precipitate cannot be due to the presence of ordinary chlorine, because a solution of chlorine has a characteristic smell, and also bleaches litmus paper. These properties are not possessed by a solution of sodium chloride. Nor is the white precipitate given by all compounds containing chlorine : neither chloroform, $CHCl_3$, nor carbon tetrachloride CCl_4 gives a precipitate. By the same reasoning as before, we see that the precipitate is given only in solutions, and must be due to some allotropic modification of chlorine which we shall represent by Cl'.

From all this, then, we can say that when hydrogen chloride is dissolved in water, there is formed $H^{\cdot}$ and Cl'; and these two parts behave quite independently, *i.e.*, each gives its own individual reactions, no matter what other substances may be present. But if this is so, then instead of the one molecule HCl we have now got the two molecules

$H^{\cdot}$ and Cl'. and, therefore, the osmotic pressure should be twice as great as would ordinarily be expected. If we use very dilute solutions, this is found to be the case.

Some further insight into the nature of solutions can be obtained in another direction, viz., from the electrical behaviour of solutions. I have here a vessel containing distilled water. When I place in the water two platinum electrodes connected with a battery and a galvanometer, no indication of a current is given. If I add cane sugar or alcohol to the water, there is still no current. If, however, I add a little hydrochloric acid, or sodium chloride, you see at once that the needle of the galvanometer is deflected, which shows that now a current is passing through the solution. In the same way other substances may be tried, and we should find that those substances which, when dissolved in water, yield conducting solutions, are exactly those substances which give solutions having an abnormally high osmotic pressure; whereas, those substances which give normal osmotic pressure do not yield conducting solutions. The two phenomena appear, therefore, to be connected.

When an electric current passes through a solution, even the most superficial observation teaches us that a liquid conductor differs from a metallic one. In the latter case, no apparent change may take place, but in the former there is a very obvious decomposition of the dissolved substance. In the case of a solution of copper sulphate, copper is deposited on one of the electrodes; in the case of sodium chloride, sodium is liberated at one of the electrodes, and then immediately decomposes the water with evolution of hydrogen and the formation of caustic soda, which gives an alkaline reaction; and chlorine is set free at the other electrode, and yields a solution having bleaching properties. Whenever a current passes through a conducting solution, therefore, there is a movement of ponderable particles through the liquid, some moving towards the one electrode, others towards the other electrode.

This conclusion was reached many years ago by Faraday, who called the moving particles which thus conveyed the electricity through the solution, *ions*; and this term is still employed.

Without attempting to trace the development which the ideas regarding conduction in solutions underwent during the middle decades of last century, I state at once the theory which is coupled with the name of Arrhenius, and which is the most satisfactory theory regarding the constitution of conducting solutions which has so far been propounded. This theory states that when an electrolyte* is dissolved in water, the molecules undergo to a greater or less extent, dissociation into ions, or oppositely (electrically) charged particles, which lead an independent existence in the solution, have their specific properties and reactions, and behave, therefore, as independent molecules. When two electrodes are placed in a solution of an electrolyte and connected with a battery, then the positively charged electrode (called the *anode*) attracts the negatively charged ions (the *anions*); and the negatively charged electrode (the *cathode*) attracts the positively charged ions (the *cations*). These ions move in opposite directions through the solution, and give up their charges at the electrodes, and it is their movement through the solution that constitutes the electric current in the solution.

With regard to the nature of the different ions, we can say that when a salt ionises in solution, the metal part forms the cation, and the acid part the anion. Thus, for example, sodium chloride, NaCl, ionises into the positively charged cation Na^+, and the negatively charged anion Cl^-. (Instead of using the plus and minus sign to represent the charges, it is more convenient and more usual to employ a dot to represent a positive, and a dash to represent a negative charge. Thus, we should write the ions of sodium chloride, $Na^{\cdot}$ and Cl'). In the case of acids, hydrogen forms the cation, so that hydrochloric acid, for example, gives the ions $H^{\cdot}$ and Cl'; nitric acid, the ions $H^{\cdot}$ and NO_3'. It is indeed, to the presence of the hydrogen ion that the so-called acid properties are to be ascribed. In the case of alkalis, *e.g.*, sodium hydroxide, NaOH, the anion is formed by the hydroxyl group. Thus, NaOH ionises into $Na^{\cdot}$ and OH'. It is to the presence of this hydroxyl ion that the general properties of alkalis are due.

---

* An electrolyte is a substance which, when dissolved, yields a conducting solution.

Even at the first glance it will be seen that this theory of ionisation, or electrolytic dissociation, enables us to co-ordinate a number of different phenomena, such as the chemical properties of electrolytes, their behaviour as regards osmotic pressure in solution, and their electrical behaviour. Cane sugar in solution, although it contains hydrogen, does not contain hydrogen ion, and therefore does not have an acid reaction. That it is not ionised is shown by the fact that it does not conduct electricity. Similarly, a solution of chloroform (in alcohol) does not conduct electricity; it is, therefore, not ionised, and so does not give a precipitate with silver nitrate.*

So far we have spoken only of binary electrolytes, *i.e.*, electrolytes which are capable of yielding only two ions. But a molecule of sodium sulphate, for example, is capable of yielding three ions, viz., two sodium ions and one sulphate ion; and there are other salts, again, capable of yielding more than three ions. Now it is evident that since a solution of sodium sulphate, to keep to that example, is, as a whole, neither positively nor negatively charged, the amount of electricity associated with one SO_4-ion must be equal to that associated with two Na-ions. But the amount of electricity associated with a gram-ion (weight of ion in grams) of sodium is 96580 coulombs, and as this is the smallest amount of electricity associated with the gram-ionic weight of any substance, it is taken as the unit charge. Hence, the symbol for sodium ion is written with only one plus sign or one dot. But since the SO_4-ion must carry twice as great a charge, we must write it with two minus signs, or with two dashes, thus—SO_4''. We can also have cations carrying more than one unit of charge; thus copper sulphate gives the ions $Cu^{\cdot\cdot}$ (one gram-ion, 63 gm., being associated with 2 × 96580 coulombs) and SO_4''. In general the number of unit charges which a gram-ion carries is given by the valency of the ion; thus we have the trivalent ions $Fe^{\cdot\cdot\cdot}$, $Al^{\cdot\cdot\cdot}$, etc., and the ion PO_4''' of phosphoric acid (H_3PO_4). This, now, allows us to understand Faraday's law of electrolysis, which states that when a current is passed through solutions of salts of different metals, the amounts of these set free at the electrodes are proportional to their atomic weights divided by the valency. Thus, the current which will liberate 1 gm. of hydrogen, or 23 gm. of sodium, will deposit only $\frac{63}{2}$ gm. of copper. For we have seen that every 23 gm. of sodium as ion are associated with 96,580 coulombs of electricity; and if this amount is passed through a solution of a sodium salt, 23 gm. of sodium will be deposited. But every 63 gm. (1 gram-atom) of copper in the ionic state is associated with 2 × 96580 coulombs, and therefore if we pass only 96580 coulombs through the solution, only $\frac{63}{2}$ gm. of copper will be deposited.

---

* It ought to be mentioned that the converse of this does not hold; immediate interaction between substances accompanied by precipitation *may* occur even when there is no appreciable conductivity and therefore no appreciable ionisation. These cases are, however, comparatively rare.

Degree of Ionisation.—Although, according to the theory of Arrhenius, the molecules of salts are capable of breaking up into ions, it does not necessarily occur that all the molecules thus break up. It is therefore necessary to determine the degree of dissociation or ionisation, *i.e.*, the relative number of the molecules which yield ions. This, we shall learn, varies with varying conditions, more especially with varying concentration.

The first method which may be employed to determine the degree of ionisation is the cryoscopic. The depression of the freezing point enables us to state the number of independent individuals in the solution, *i.e.*, the number of undissociated molecules *plus* the ions. We have already learned that the isotonic coefficient gives the ratio of the observed to the calculated depression of the freezing point; or, since the depression of the freezing point is proportional to the number of dissolved molecules, the isotonic coefficient gives the ratio of the number of independently acting molecules to the number calculated from the ordinary molecular weight of the dissolved substance. Thus, for example, a solution of sodium chloride containing 0.20 gram-molecules per litre, gives a depression of the freezing point of 0.690°. But, normally, a solution containing 0.20 gram-molecules per litre should give a depression of only 0.370°. Therefore, the actual or effective number of gram-molecules per litre is 0.690/0.370=1,865. From this we can calculate the extent to which the sodium chloride is ionised in the solution. Each molecule of NaCl is capable of giving two new molecules or ions. If we dissolve, say, 1 mole of sodium chloride, then, in solution, the effective number of gram-molecules will be two, provided the ionisation is complete; and the depression of the freezing point would be twice as great as when no ionisation occurs. Suppose that only a certain fraction, x, of the NaCl molecules are ionised, then there would be $(1-x)$ gram-molecules of unionised NaCl, and $2x$ gram-molecules of ions. In all, therefore, there would be $1-x+2x=1+x$ gram-molecules (since an ion has the same effect as an ordinary molecule). Hence, $i=\frac{1+x}{1}$ or $x=i-1$. But, in the above example, $i=1.865$; therefore, $x=0.865$. Of the total number of molecules of sodium chloride dissolved, therefore, 0.865 of them undergo ionisation. Similarly, if we have a salt, say potassium sulphate, K_2SO_4, capable of giving three ions, then the effective number of gram-molecules is increased in the ratio of $1:(1-x)+3x$ or as $1:1+2x$. Hence, $x=\frac{i-1}{2}$. Or, in general, if the salt molecule can yield n ions, the degree of ionisation will be given by $x=\frac{i-1}{n-1}$. When $i=n$, then $x=1$, and the ionisation is complete.

Now, on determining the freezing points of solutions of different concentration, it is found that the value of i increases as the solution becomes more dilute, as is shown in the following table :—

TABLE 8.

CHANGE OF i WITH DILUTION, SOLUTIONS OF SODIUM CHLORIDE.

Molar concentration.	Depression (observed).	Depression (calculated).	i	x
0.200	0.690°	0.370°	1.865	0.865
0.171	0.592°	0.316°	1.871	0.871
0.169	0.590°	0.313°	1.887	0.887
0.150	0.539°	0.278°	1.942	0.942
0.127	0.464°	0.235°	1.974	0.974

For the physiologist, however, it is the calculation, not so much of the degree of ionisation as of the isotonic coefficient that is of importance. That is to say, if a salt solution of known concentration is given, it is important to be able to calculate the osmotic pressure of the solution. In order to do this, the value of x, which can be determined by quite a different method (see later), can generally be obtained from tables, and then, by means of the equation previously given, $i = 1 + (n - 1) x$, the value of i can be calculated, and hence, also, the value of the osmotic pressure.

To take an example: In a solution of NaCl containing 0.50 mole per litre (approximately 2.9 per cent.), the degree of ionisation is 0.73. From this, since $n=2$, $i=1+0.73=1.73$.

Hence, the osmotic pressure of this solution will be 1.73 times the normal. But the normal osmotic pressure at 0° would be 11.2 atmospheres; therefore, the osmotic pressure of the above salt solution will be $11.2 \times 1.73 = 19.4$ atmospheres, and the depression of the freezing point instead of being 0.925°, will be $0.925 \times 1.73 = 1.600°$.

While cryoscopic measurements yield directly the effective concentration of a solution, *i.e.*, the sum of the concentrations of unionised molecules plus ions, and indirectly the concentration of the ions, the latter can be determined more easily by an electrical method. We may give the principle of this method without entering too much into detail.

By conductivity one means the reciprocal of the resistance, which is the factor directly measured. By *specific conductivity* is meant the conductivity of a cube of 1 cm. side; but since the whole of the solution does not take part in the conduction, it is better to compare the conductivity not of a centimetre cube of solution, but the conductivity of solutions containing equivalent amounts of dissolved substance between the electrodes. This is known as the *equivalent conductivity*.

Now, the connection between the specific and the equivalent conductivity is obtained in the following way. Suppose we have a rectangular vessel, the two opposite sides of which are of metal and 1 cm. apart. If we place 1 c.c. of solution in such a vessel, then the conductivity measured will, of course, be the specific conductivity. Further, suppose that there were 1 gm. equivalent of dissolved substance in the 1 c.c. of solution, then the conductivity measured would also be the equivalent conductivity. Now dilute the solution by pouring in distilled water, making the volume up to, say, 1000 c.c. or 1 litre. The conductivity of this solution will now be 1000 times the specific conductivity, because there are now, so to speak, 1000 cubes of solution of 1 cm. side. But since we have still 1 gm.-equivalent of dissolved substance between the electrodes, the conductivity which is measured is also the equivalent conductivity; so that in this case the equivalent conductivity is 1000 times the specific conductivity. Or, in general, we can say, the equivalent conductivity of a solution is equal to the specific conductivity multiplied by the volume in cubic centimetres in which 1 gm-equivalent of the electrolyte is dissolved.

On carrying out the necessary measurements, it is found that the *equivalent* conductivity of all solutions of electrolytes increases with dilution. This can be shown very easily by means of the rectangular vessel already described. If we place in this cell a certain quantity of a solution of an electrolyte, *e.g.*, ammonium hydroxide, and connect the two electrodes with a battery, we observe that the needle of the galvanometer is deflected to a certain extent, indicating the passage of a certain current through the solution. If, now, I pour water into the cell so as to dilute the solution, you see that the galvanometer needle is deflected to a greater and greater extent, showing, therefore, that the current passing through the solution is becoming greater. Since the driving force (the electromotive force of the battery) remains unchanged, it follows that the resistance of the solution has diminished with dilution, or the conductivity has increased.

This increase in the conductivity must be due to one or other of two factors, or to both of them, viz., to an increase in the number of ions, or particles carrying the current, or to an increase in their velocity of movement. It has, however, been found that dilution has very little influence on the velocity of movement of the ions, and so we must conclude that the increase in the conductivity is due to an increase in the number of ions. We also obtained this result by means of cryoscopic measurements (Table 8).

But the increase in the number of ions cannot go on indefinitely; it must cease when all the molecules have become ionised. Hence, the equivalent conductivity is found to increase with dilution only up to a certain point, beyond which dilution has no longer any effect; and there is therefore a certain maximum value of the equivalent conductivity, which depends on the electrolyte. This increase in the equivalent conductivity with dilution is shown very clearly by the following table :—

TABLE 9.

EQUIVALENT CONDUCTIVITIES AT 18°.

Dilution in litres.	KCl	$\frac{1}{2}K_2SO_4$	$\frac{1}{2}MgSO_4$
1	98.2	71.8	28.9
10	111.9	95.9	50.1
100	122.5	117.4	76.6
1000	127.6	129.0	100.2
2000	128.3	130.8	104.8
5000	129.1	132.7	108.7
10000	129.5	133.5	110.4

But we have already learned that the conductivity of a solution is practically proportional to the concentration of the ions. If we represent the equivalent conductivity of a solution at very great dilution (so-called infinite dilution) when all the electrolyte is ionised, by C_∞, and the equivalent conductivity at any other dilution, v litres, when only the fraction x of the electrolyte is ionised, by C_v, then it follows that $Cv = x\ C_\infty$, and therefore $x=\frac{C_v}{C_\infty}$. Hence, we can determine the degree of ionisation at any given dilution by measuring the equivalent conductivity at that dilution, and dividing it by the equivalent conductivity at infinite dilution when all the electrolyte is ionised.

With regard to the degree to which different electrolytes are ionised, it is found that, in general, all salts are largely ionised, but that in the case of acids and bases there are great differences, some acids and bases, *e.g.*, HCl and NaOH, being very largely ionised in solutions of moderate concentration, while others, *e.g.*, acetic acid and ammonium hydroxide, are only slightly ionised. This is seen from the following table, which applies to solutions containing 1 gm-equivalent in 2 litres.

TABLE 10.

	Degree of ionisation.		Degree of ionisation.
NaCl	0.74	HCl	0.85
KCl	0.79	H_2SO_4	0.53
NaAc	0.64	HAc	0.006
Na_2SO_4	0.54	NaOH	0.79
		NH_4OH	0.006

The halogen salts of mercury are among the more important exceptions to the rule that salts are largely ionised.

SOME APPLICATIONS OF THE IONIC THEORY.

As an effect due to the action of ions, we may again refer to the production of artificial parthenogenesis. The ova of chaetopterus, for example, undergo parthenogenic development under the influence of small quantities of potassium ion, *i.e.*, addition of any potassium salt causes the development of the eggs; but other salts, which do not yield potassium ion, are without influence. Similarly, the ova of the starfish can be caused to undergo parthenogenic development by the hydrogen ion—by the addition, therefore, of any acid—but not by potassium ion. Again, the ova of the amphitrite undergo parthenogenic development under the influence of calcium ion, but not under the influence of potassium or of hydrogen ion. Finally, it was found that the ions, $Mg^{\cdot\cdot}$, $Sr^{\cdot\cdot}$, $Li^{\cdot}$, $Na^{\cdot}$, $K^{\cdot}$, are without action on them.

A number of very interesting observations have also been made by Loeb and others on the effect of potassium and sodium salts in promoting the production of dwarf and giant embryoes. In presence of sodium chloride, for example, it was found that there was a tendency for more than one embryo to be formed from one ovum—the embryoes being then smaller than usual. In presence of potassium chloride, however, there is a tendency towards agglutination of ova, which then undergo development with the production of giant embryoes. This difference in the action of potassium and sodium ions is of considerable interest.

The chemotactic action of ions has also been observed very clearly in the attraction of the spermatozoa to the archegonia of the ferns. It was found by Pfeffer and by Buller that the negative ion of malic acid ($C_4H_4O_5''$) has the greatest attractive power. Since this ion is given by any of the salts of malic acid, the same effect is observed, irrespective of the cation which is present, provided the concentration of the malate anion is the same. Owing to the difference in the degree of ionisation of the different salts, equivalent solutions will not necessarily have the same influence, so that it is necessary to take the degree of ionisation into account. Other ions were also found to have an attractive force, but the hydrogen ion apparently repels, so that in free malic acid the attractive influence of the malate anion is counteracted by the repulsive effect of the hydrogen ion.

Toxic Action of Ions.—One of the best illustrations of the influence of ions is found in the study of the disinfective and toxic action of ions, which is observed very clearly, and has been well investigated in the case of mercury salts. The experiments were carried out chiefly with mercuric chloride, bromide, and cyanide, the test being the poisonous action of equivalent solutions of these salts on the spores of the anthrax bacillus. From conductivity measurements, it is found that these salts are not ionised to any great extent; but the ionisation of the chloride is greater than that of the bromide, and this in turn is more ionised than the cyanide. In fact, the cyanide appears to be practically unionised. If, then, the toxic action of the above salts is due to the mercury ion ($Hg^{\cdot\cdot}$), it would be expected that the action of the chloride would be greater than that of the bromide, and that this would be more effective than the cyanide. This was found to be the case, as can be seen from the following table :—

TABLE 11.

Solution.	After treatment for 20 minutes there developed	After treatment for 85 minutes there developed
1 mole $HgCl_2$ in 64 lit.	7 colonies	0 colonies
1 ,, $HgBr_2$ in 64 lit.	34 ,,	0 ,,
1 ,, $Hg(CN)_2$ in 16 lit.	∞ ,,	33 ,,

From this we see, in the first place, that the toxic action increases with the time during which the solutions are allowed to act on the spores; and, secondly, the toxic effect runs parallel with the degree of ionisation of the salts. This shows that the toxic action depends chiefly on the concentration of the mercury ion, although it is not at all improbable that the chloride and bromide ions have also some, although comparatively slight, influence. The effect of the neutral molecules, also, appears to be very slight, if, indeed, they exercise any influence at all.

Similar results are obtained with the salts of silver, but as these are more highly ionised, the effect of the anion makes itself felt. Thus :—

TABLE 12.

Solution.	Number of colonies which developed after treatment for 60 minutes.
1 mole $AgNO_3$ in 20 lit.	27
1 ,, $AgClO_3$ in 20 lit.	42
1 ,, $AgClO_4$ in 20 lit.	219

Since the above salts are ionised to approximately the same extent, it would appear that the nitrate ion is more toxic than the chlorate and perchlorate ions.

The theory of ionisation, however, not only helps us to understand the action of the salts, but it also suggests methods of overcoming the toxic action. If this is due to some particular ion, then we should be able to diminish or to annul the action by diminishing the concentration of that ion. The first, most obvious way of doing this is to diminish the degree of ionisation of the salt.

Let us consider only the case of mercuric chloride or corrosive sublimate. This salt undergoes ionisation as expressed by the equation, $HgCl_2 = Hg^{\cdot\cdot} + 2Cl'$. At any given concentration and temperature, there is a certain condition of equilibrium between the unionised mercuric chloride and the ions $Hg^{\cdot\cdot}$ and Cl'. Now, we can diminish the degree of ionisation, and therefore the concentration of $Hg^{\cdot\cdot}$, by adding chloride ions, in accordance with what is known as the Law of Mass Action. As this is a very important law, we shall make a slight digression here in order to give a short account of it.

Law of Mass Action.—You know that a chemical reaction is generally represented in the form of an equation, *e.g.*, $Zn + H_2SO_4 = ZnSO_4 + H_2$, meaning that if we allow 1 mole (65 gm.) of zinc to act on 1 mole (98 gm.) of sulphuric acid, there are produced 1 mole (161 gm.) of zinc sulphate, and 1 mole (2 gm.) of hydrogen. In this case the reaction takes place completely from left to right, *i.e.*, all the zinc is converted into zinc sulphate. But there are many reactions which do not take place completely as represented by the chemical equation, but which cease before all the reacting substances have been used up. The usual reaction which is quoted as an example of this is the action between acetic acid and alcohol. If these two substances are mixed together, there are formed ethyl acetate and water, as represented by the equation, $CH_3{\cdot}COOH + C_2H_5OH = CH_3{\cdot}COOC_2H_5 + H_2O$.

In this case, however, we should find that if we take equivalent quantities, say 1 mole, of the acid and alcohol, the reaction would not take place completely, *i.e.*, the acid and the alcohol would not entirely disappear, but only two-thirds of them; while one-third would remain unchanged, no matter how long they were left in contact. This is due to the fact that water acts on the ethyl acetate, converting it back again into acetic acid and alcohol. In other words, the reaction tends to be reversed according to the above equation read from right to left. We have therefore two opposed reactions taking place, and the consequence is the production of a condition of equilibrium between the acid and alcohol on the one side, and the ethyl acetate and water on the other. We have, therefore, what is known as a balanced reaction, and to represent this the usual sign of equality is replaced by two arrows, so that the interaction between acetic acid and alcohol should be written

$$CH_3{\cdot}COOH + C_2H_5{\cdot}OH \rightleftarrows CH_3{\cdot}COOC_2H_5 + H_2O.$$

Now, in the case of all balanced reactions, the law of mass action states that the product of the concentrations (expressed in gram-molecules per litre) of the substances on the one side, divided by the product of the concentrations of the substances on the other side of the arrows, is equal to some definite, constant number (provided the temperature remains unchanged). This law is expressed mathematically by the equation

$$\frac{C_a^{n_1} \times C_b^{n_2} \times \dots}{C_x^{n_3} \times C_y^{n_4} \times \dots} = \text{constant},$$

in which C_a, C_b, etc., represent the concentrations of the substances on the one side of the chemical equation, and C_x, C_y, etc., represent the concentrations of the substances on the other side of the equation. The indices are the number of molecules of the different substances reacting. In the case of the formation of ethyl acetate from acetic acid and alcohol, the indices are all unity, so that the condition of equilibrium is given by the expression :

$$\frac{C_{\text{acid}} \times C_{\text{alcohol}}}{C_{\text{ester}} \times C_{\text{water}}} = \text{constant}.$$

The value of the constant must, of course, be obtained by experiment; in the case just mentioned it is equal to $\frac{1}{4}$.

Now, it does not at all matter in what proportions we mix the four substances, acetic acid, ethyl alcohol, ethyl acetate and water; the same condition of equilibrium is always attained, viz., that represented by the immediately preceding equation. Suppose, then, that when equilibrium has been attained, a further quantity of acetic acid is added, we thereby increase the concentration of one of the components of the mixture; the product of concentrations in the numerator is thus increased, and, in order that the above equation shall remain true, the value of the denominator must also increase. That is to say, there must be a further combination of alcohol and acid to form ester and water. By adding sufficient acid, we can then cause all the alcohol to be converted into ester.

We can at once apply what we have just learned to the case of mercuric chloride, and the diminution of the degree of ionisation of this salt by addition of sodium chloride.

When mercuric chloride is dissolved in water, ionisation takes place to a limited extent, and an equilibrium is produced between the unionised mercuric chloride and the ions, as represented by the equation: $HgCl_2 \rightleftarrows Hg^{\cdot\cdot} + 2\,Cl'$.

According to the law of mass action, however, we can say that

$$\frac{\text{Conc}_{\text{HgCl}_2}}{\text{Conc}_{\text{Hg}^{\cdot\cdot}} \times \text{Conc.}^2_{\text{Cl}'}} = k.$$

in which *k* has a definite numerical value, at any given temperature, no matter how the concentrations of the unionised salt or of the ions may be altered. Hence, if we increase the concentration of the chloride ions by the addition of, say, sodium chloride (which yields the ions $Na^{\cdot}$ and Cl'), the result will be an increase in the concentration of the unionised mercuric chloride. In other words, a certain amount of the mercuric ions must combine with chloride ions to form unionised salt, so that, as a result, the concentration of the mercuric ions must diminish. It will also be evident that, in order to effect the diminution of concentration of mercuric ions, it is not necessary to add sodium chloride; any chloride capable of yielding the chloride ion will serve the purpose.

The diminution in the toxic power of mercuric chloride produced by the addition of sodium chloride is shown in the following table :—

TABLE 13.

16 litres of solution contained	After treatment for 6 minutes there developed
1 mole $HgCl_2$	8 colonies
1 „ $HgCl_2$ + 1 mole NaCl	32 „
1 „ $HgCl_2$ + 2 moles NaCl	124 „
1 „ $HgCl_2$ + 4 „ NaCl	382 „
1 „ $HgCl_2$ + 10 „ NaCl	1087 „

Whenever, therefore, the toxic action of a salt is due to the cation, we can diminish the toxicity by adding a non-toxic salt having the same anion.

But there is another way in which the concentration of an ion can be diminished, namely, by causing it to enter into combination, so as to form what is known as a complex ion. Thus, for example, the toxic action of silver salts can be reduced by the addition of sodium thiosulphate, $Na_2S_2O_3$. The reason of this is that the silver ion combines with the thiosulphate ion (S_2O_3'') to form the complex AgS_2O_3', which is non-toxic. In this way the toxic silver ions can be removed from the solution.

This formation of complex ions also plays a part in the reduction of the toxic action of mercuric chloride by addition of sodium chloride, as some of the mercury ions combine with the chloride ions to form the ion $HgCl_4''$ which is non-toxic.

From all that has been said, it will be evident that in estimating the toxic action of salt solutions, it is not enough to determine merely the total concentration of dissolved salt, but also the concentration of the ions. It must, however, be stated that the question is not in all cases so simple as it would appear from the foregoing to be; but complications are met with, such as influence of diffusibility, and others which have not all been satisfactorily cleared up.

The effect of acids and alkalis (*i.e.*, of the hydrogen and the hydroxyl ions) on the growth of seedlings has also been investigated, and the same general relationships found between the degree of ionisation and the degree of activity.

It has also been found that ions exercise an influence on the solubility of substances in water, *e.g.*, of phenol, carbon dioxide, nitrous oxide, etc. This is of interest as affording an explanation of the effect of addition of sodium chloride in increasing the toxicity of solutions of phenol (carbolic acid). This effect is shown by the following table, which gives the number of colonies which developed after treatment with different solutions.

TABLE 14.

	Duration of action on anthrax bacillus in days.								
Solution.	0	1	3	7	9	11	13	15	
3% Phenol	6300	1390	1260	950	810	530	560	530	col.
,, ,, +1% NaCl	5720	1450	1320	360	—	—	100	0	,,
,, ,, +8% NaCl	1940	150	50	0	—	—	—	—	,,

This behaviour is probably due to an alteration in the distribution of the phenol between the water and the cells (bacteria); addition of salts diminishing the concentration of the phenol in the water and increasing it in the cells. Other electrolytes give a similar result, but unionised salts appear to have comparatively little action.

PERMEABILITY OF MEMBRANES AND ABSORPTION.

WHEN treating of osmotic pressure we regarded the membrane as being impermeable to dissolved substances, although mention was made of the fact that the walls of the red blood corpuscles are permeable to some salts but not to others. Investigation, however, has shown that the permeability of certain animal membranes is essentially selective; that they are permeable to certain ions but not to others. It is probable that this plays an important *rôle* in certain cases of absorption and secretion, and that it affords an explanation of a number of interesting phenomena.

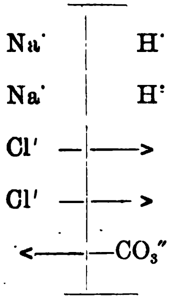
In considering the permeability of membranes to ions, it must, in the first place, be borne in mind that an ion can pass through a membrane only when it is accompanied by another ion of opposite sign (cation accompanied by anion), or when its place is taken by another ion of the same sign from the opposite side of the membrane. The reason of this is of course that the ions are highly charged with electricity, and there cannot be an excess of free positive or negative ions in the solution. The passage of a cation through a membrane which is impermeable for the anion would be resisted by the attraction between the electrical charges.

As an example of a membrane which is permeable only for certain ions, we may take the walls of the red blood corpuscles. These allow Cl' to pass, but not $Na^{\cdot}$. To this fact is due the remarkable behaviour that when blood is saturated with carbon dioxide, it becomes more alkaline. The explanation can be given as follows. In the normal state, the wall of the blood corpuscle is permeable to the chloride ion but not to the sodium ion, and for this reason, the passage of the chloride ion will be prevented. Suppose, however, that carbon dioxide is passed into the blood. A certain amount of the carbon dioxide will combine with the water to form carbonic acid, which then dissociates into ions in accordance with the equations

$$H_2CO_3 = H^{\cdot} + HCO_3' \text{ and } HCO_3' = H^{\cdot} + CO_3''$$

There is therefore a production of hydrogen ion, and as the walls of the corpuscles are permeable to this, the passage of the chloride ion is also rendered possible since it can be accompanied by the hydrogen ion. As carbonic acid is only very slightly ionised, the amount of hydrogen ion present at the beginning will be very small. In proportion as hydrogen ion passes away through the wall of the corpuscle, however, more is formed by the dissociation of the carbonic acid (in accordance with the law of mass action), so that ultimately we shall have $Na^{\cdot}$ and CO_3'' left behind. But as carbonic acid is a weak acid (*i.e.*, is only slightly ionised), part of the CO_3'' will combine with hydrogen ions formed by the ionisation of water, and there will thus be produced an excess of free hydroxyl ions; that is to say, we get the following process occurring :—$CO_3'' + 2H^{\cdot}OH' = H_2CO_3 + 2OH'$ Owing to the presence of excess of hydroxyl ions, the liquid reacts alkaline.

In an analogous manner, we can explain the fact that when red blood corpuscles are saturated with carbon dioxide and then placed in a salt solution, the solution becomes alkaline. The process which takes place here is represented by the diagram. Within the corpuscle, there will be formed hydrogen ions and carbonate ions. Since the membrane is permeable for chloride ions and for carbonate ions, the former can pass inwards into the corpuscle, because now their place can be taken by the carbonate ions which pass out into the salt solution. Outside the corpuscles, therefore, we again have sodium ions and carbonate ions, and consequently, as explained above, the solution acquires an alkaline reaction.



Hydrolysis. We have just seen that when normal sodium carbonate, Na_2CO_3 is dissolved in water, the solution reacts alkaline. This, at first sight, strange behaviour can be easily explained on the basis of the electrolytic dissociation theory; and as the phenomenon is not infrequently met with, a somewhat fuller explanation may be given.

When dealing with the theory of ionisation, the solvent water was regarded as a neutral medium in which salts dissociate into ions. But water is itself ionised, although to a very slight extent, into hydrogen ions, and hydroxyl ions, and these ions are capable of interacting with other ions formed in the solution. Suppose, for example, that sodium acetate is dissolved in water. The salt dissociates into the ions $Na^{\cdot}$ and $C_2H_3O_2'$ (which may be represented by Ac'), and since the degree of ionisation is large (as in the case of almost all salts), there will be a considerable concentration of acetate ions in solution. But acetic acid is a weak acid; that is to say, it is only slightly ionised, and, consequently, according to the law of mass action, the product of concentrations of hydrogen ion and acetate ion can only be a small fraction of the concentration of the undissociated acetic acid. A certain proportion of the acetate ions will therefore combine with hydrogen ions produced by the ionisation of the water. Since sodium hydroxide is a strong base, *i.e.*, is largely ionised, there will be practically no combination of the sodium ions and the hydroxyl ions also formed by the ionisation of the water, and consequently there will be free hydroxyl ions in solution. These free hydroxyl ions are the cause of the alkaline reaction.

Now, we see from this that the reason why a solution of sodium acetate or of sodium carbonate reacts alkaline is that the acid from which the salt is formed is a weak or slightly ionised one, whereas the base was a strong one, so that there was very little tendency for the metal ion and the hydroxyl ion to combine to form undissociated base. Consequently, we shall expect, and we find, that all salts which are formed from a strong or highly ionised base, and a weak or slightly ionised acid, when dissolved in water yield solutions which have an alkaline reaction. Further, it is evident that the alkaline reaction will be all the greater, the weaker the acid is, because then a larger amount of the anion will combine with hydrogen ion to form unionised acid, and the concentration of the hydroxyl ion will be all the greater. Hence we find that a solution of potassium cyanide is strongly alkaline in reaction, because hydrocyanic acid is very slightly ionised; consequently, a considerable amount of the cyanide ion combines with hydrogen ion, and an equivalent amount of hydroxyl ion is left free in solution along with the potassium ion. In the case of potassium cyanide, the degree of hydrolysis amounts to between 1 and 2 per cent.

An analogous behaviour is found in the case of salts formed from weak bases and strong acids. In this case, the solution will have an acid reaction, because the cation of the salt will combine with hydroxyl ion derived from the water, to form undissociated base, and free hydrogen ions will be left in the solution along with the anions of the salt. Since the acid is strong, there will be little tendency for these ions to combine.

The production of an acid or alkaline reaction owing to the interaction of the ions of water with the ions of the dissolved salt, is known as *hydrolysis*; and in dealing with the behaviour of solutions, it is very necessary to bear this behaviour in mind.

Absorption from the Stomach. The stomach we can regard as a sack, the walls of which are surrounded by a liquid, the blood plasma. If a salt solution having a higher osmotic pressure than the plasma is passed into the stomach, the result of the difference of the osmotic pressure will be that either water will pass into the stomach, if the walls are impermeable for the dissolved salt, or salt will pass out from the stomach, if the wall is permeable; or, of course, both these processes may occur.

It has been found that certain animal membranes possess the very important property of being permeable only in one direction. Thus, for example, it has been found that the membrane surrounding an egg will allow water to pass inwards, but not outwards; and it has also been shown that the skin of the frog, while permeable in both directions for potassium ion and chloride ion, is permeable for the sodium ion only from outside to inside. When the membrane is killed, however, it becomes permeable for all substances in either direction.

This behaviour of certain animal membranes is of importance in studying the appearance of hydrochloric acid in the stomach. The view which is found in most text-books at present is that the acid is secreted within the cells of the stomach wall and is then passed into the stomach from them. Another explanation of the appearance of this acid in the stomach can, however, be given.

In the first place it would seem that the stomach wall is permeable for water only in one direction, namely, from the outside into the stomach. Then again, in considering the permeability of the wall for salts, we must take into account the ionisation of the salts and the permeability of the stomach wall for these ions. In doing this, it is necessary to bear in mind that one ion can pass through only when accompanied by another ion of opposite sign, or when the place of the latter is taken by another ion of the same sign from the other side of the membrane.

Experiments were first made with respect to the permeability of the stomach wall for chloride ions. Dilute hydrochloric acid was passed into the stomach, and after about 50 minutes it was found that the liquid which passed into the duodenum had the same concentration of chloride ion, but only half the concentration of hydrogen ion. Evidently, therefore, the stomach wall is permeable for hydrogen ion but not for chloride ion. But the place of the hydrogen ions which passed through the stomach wall must have been taken by some other cation from the blood, *e.g.*, sodium ion. This permeability of the stomach wall for sodium and hydrogen ions, but not for chloride ions, is of importance for the question which we have raised.

If a solution of sodium chloride is passed into the stomach it is found that water passes in from the outside, and that after a time, a portion of the sodium chloride has disappeared from the stomach. After about 50 minutes hydrochloric acid is found in the stomach, and the osmotic pressure of the blood plasma gradually rises. An explanation of this behaviour can be given as follows. Since the wall of the stomach is impermeable for chloride ions, neither these nor the sodium ions can at first pass through into the blood, but only the unionised sodium chloride. But the sodium ion can pass through provided its place is taken by another cation, passing through in the opposite direction. And this is apparently what occurs, the cations which pass into the stomach being hydrogen ions from the blood. Do hydrogen ions, then, exist in the blood? We have already learned that water is ionised to a certain small extent into hydrogen ions and hydroxyl ions, and as blood has been shown to be a neutral liquid, it follows that the concentration of hydrogen ions in the blood will be the same as that in pure water. Although this concentration is very small, it does not make it impossible for the hydrogen ions to pass into the stomach and be present there in greater concentration than in the blood. Because, suppose hydrogen ions to pass into the stomach while their place is taken by sodium ions, then the equilibrium

$$H_2O = H^{\cdot} + OH'$$

is disturbed, and more water must ionise, yielding a fresh amount of hydrogen ions. In this manner the acidity of the stomach is increased owing to the increased concentration of hydrogen ions, and at the same time, the blood becomes more and more alkaline, owing to the increased concentration of hydroxyl ions.

According to this explanation, then, the hydrochloric acid is not secreted as such by the cells of the stomach wall, but is produced owing to the wandering away of the sodium ions and their replacement by hydrogen ions. Its formation, therefore, depends on the presence of an anion (Cl') which cannot pass outwards through the stomach wall. If free chloride ions are absent, we should expect that no hydrochloric acid will be found in the stomach contents. Thus if sugar solution is introduced into the empty stomach and care is taken that chloride ions are prevented from entering, say from the saliva, it is found that the cell contents retain a neutral reaction, even although water passes freely from the wall into the stomach. This shows that the mere presence of food-stuffs in the stomach is not sufficient to give rise to the formation of hydrochloric acid. So soon, however, as free chloride ions are introduced into the stomach, hydrochloric acid ($H^{\cdot}$) begins to make its appearance. Even when there is the normal amount of chlorides in the blood, and even when hydrochloric acid is injected, no hydrochloric acid appears in the stomach, if the contents did not contain chloride ions.

If the above view is correct, the presence of hydrochloric acid in the stomach is due to the permeability of the cell-walls for sodium ions and hydrogen ions, and its impermeability for chloride ions. Similarly, there will be a production of acid in the stomach if there be introduced into the stomach the sodium salts of any other acid, the anion of which cannot pass through the cell-walls, *e.g.*, sodium bromide, sodium iodide, etc.

Absorption from the intestine. With regard to absorption from the intestine, the opinion of physiologists is very varied. Cohnheim emphasises the difference in the behaviour of living and dead intestinal epithelium, with respect to permeability. If, for example, a sugar solution is introduced into the living intestine, it is absorbed, but no sodium chloride or other constituent of the plasma passes into the intestine. This occurs, however, if the epithelial cells are killed with arsenic, or with sodium fluoride. Cohnheim ascribes, therefore, to the normal healthy intestinal epithelium the property of semi-permeability in one direction, such as we have seen to be possessed by the skin of the frog with respect to sodium ions.

Waymouth Reid carried out measurements on the effect of intra-intestinal pressure on absorption, and found that even when the intra-intestinal pressure fell below that of the capillaries, absorption still took place. His explanation of absorption, however, is not the same for different substances; in the case of salt solutions, it is referred to physiological forces, while in the case of glucose, it is referred chiefly to diffusion.

Here, as in all cases, Hamburger seeks to analyse the process into purely physical ones, although he recognises that the life of the cell can exercise an influence on the processes. In one respect, he has the superiority over other investigators in that he recognises that the process of absorption may depend on a number of factors, some of which may be more important in some cases than in others; and he considers that not only physical but also chemical influences play a part. The various factors which he considers enter into the process of absorption are : (1) intra-intestinal pressure. Increase of this, produced by breathing, peristaltic movements and weight of the intestine, increases the absorption. On lowering the pressure to zero, no evidence of absorption was observed. (2) Imbibition (or soakage, as in gelatine) by the epithelial cells. (3) The drawing power of the capillary blood stream. (4) Diffusion. (5) Osmosis. The diffusion will depend not only on the substances but also on the permeability of the epithelium, which may be very different in the case of living and of dead epithelium.

Of course, the epithelium is permeable for water outwards as well as inwards, and if the osmotic pressure of the contents of the intestine is higher than that of the blood, then water will pass into the intestine and dilute the contents. The dilution of the contents of the intestine will be increased, therefore, by the introduction of a soluble substance which does not readily diffuse out through the intestinal wall, *e.g.*, magnesium sulphate. It is to this property that the laxative action of Epsom salts is due. (?)

Urine Secretion. In the case of the secretion of urine, the possibility of a purely physical explanation of the process seems less, on account of the fact that we are here dealing with the separation of a liquid from the blood which possesses a higher osmotic pressure than the blood itself, and also contains its soluble constituents in other proportions than they are contained in the blood.

The two most commonly accepted views as to the formation of urine in the kidneys are, I believe, that of Ludwig and that of Bowman modified by Heidenhain. According to the former theory, there is a filtration of dilute urine in the capsules, and this dilute urine on passing through the urinary tubules, returns water to the blood and becomes more concentrated.

According to the theory of Bowman and Heidenhain, the whole process of the secretion of urine is referred to "cell activity," the water and salts being secreted by the glomeruli and the specific urinary constituents, urea, urates, etc., by the epithelium of the urinary tubules. This is the view which is most generally accepted at present.

The chief experiments in support of the view that the epithelium of the convoluted tubules secretes the urea and urates, are those of Nussbaum on the kidney of frogs. In this case the glomeruli are supplied by the renal artery; the tubules by the renal portal vein. Nussbaum stated that peptones and sugar are eliminated by the glomeruli, so that if the renal artery is ligatured they are not got rid of from the blood. On the other hand, urea, etc., is eliminated by the tubules, and this even when the renal arteries are ligatured.

These experiments were, however, called in question by Beddard, who showed that in Nussbaum's experiments there was imperfect ligature of the renal arteries. When the glomeruli are completely cut off by ligature of the renal arteries, no secretion of urea takes place, even if this be injected.

Again, experiments by Gurwitsch appear to show that the cells of the tubules are secretory by injecting dyes. If the renal artery is ligatured the cells become coloured; if renal portal vein is ligatured coloration of the cells is not found. Gurwitsch was also of opinion that absorption does not take place in the tubules.

Although a very large amount of attention appears to have been devoted to the study of the functional activity of the kidneys, opinion is still very divided with regard to the mode of action involved in the secretion. This is no doubt due to the conflicting nature of some of the results of the investigations which have been carried out by different physiologists.

Although attempts have been made to reduce the separation of urine by the kidneys to the action of physical forces—modified or directed by the special character of the cells—it cannot be said that a complete physical theory has as yet been advanced, although various suggestions have been made. These suggestions I will try to summarise very briefly.

The first to attempt a physical explanation of urine secretion on the basis of the doctrine of osmotic pressure, was the chemist Tammann. He adopted Ludwig's filtration theory and first sought to discover whether the epithelium of the glomeruli is semi-permeable or not; for it will depend on this whether the pressure of the blood is sufficient to cause the filtration. We can understand the principle which is involved, from the following illustration. Suppose that we have a cylinder containing a salt solution of definite osmotic pressure, enclosed at the top by a semi-permeable piston. If water is placed on the top of the piston, it will pass through the semi-permeable membrane and cause the piston to rise. In order to oppose this rise, a certain mechanical pressure must be exercised equal to the osmotic pressure of the salt solution. If the mechanical pressure is greater than this, even by a very little, water will be forced out from the solution through the semi-permeable piston. We thus obtain a filtration of the solvent through the membrane.

If, therefore, the epithelium of the glomeruli is semi-permeable, it follows that in order to cause the filtration of water, a pressure somewhat greater than the osmotic pressure of the blood will be necessary. But the osmotic pressure of the blood amounts to between six and seven atmospheres, and the blood pressure in the glomeruli is certainly not so high as that. Consequently, if we accept the view that the secretion of the urine is a filtration process, the epithelium layer cannot act as a semi-permeable membrane. If, however, it is assumed that the epithelium is permeable for crystalloids but not for colloids, *e.g.*, the proteid substances in the blood plasma, it follows that the pressure necessary to cause filtration need be only slightly greater than the osmotic pressure due to the colloids.

Tammann therefore sought to determine the osmotic pressure of the albuminoids in the plasma or serum, by means of cryoscopic measurements; and found it to be about 6 mm. of mercury. According to this, a blood pressure in the glomeruli greater than 6 mm. of mercury would be sufficient to give a filtration of urine which would contain the crystalloids but no albumin.

It may, however, also be mentioned that the osmotic pressure due to the albuminoids was also measured by Starling, by a direct method, and the value 25-30 mm. of mercury was obtained, a value which is considerably greater than that obtained by Tammann. If Starling's value is correct, then according to Ludwig's theory, a blood pressure of more than 25 mm. of mercury would be necessary. But the experiments of Magnus and Gottlieb show that caffein can cause urine secretion even when the blood pressure of the carotid is only 13-16 mm.; and it has been stated that secretion occurs even when the pressure is only 8 mm.

With regard to this, it may be said that the above argument depends for its weight on the accurate determination of the osmotic pressure of the albuminoid solution in the blood. In the cryosocopic method employed by Tammann, 0.001° corresponds to a pressure of 9 mm. of mercury, so that his value for the osmotic pressure may be wrong by several millimetres of mercury. Since it has recently been found possible with the use of thermo-electric junctions to measure depressions of the freezing point with an accuracy of about 0.000087°, a repetition of Tammann's experiments by this more refined method might be of value.

Further, according to Starling, whenever diuresis occurs, there is always hydræmic plethora, and therefore increase of pressure in the glomeruli, the volume of the kidney and the flow of urine running parallel with each other. If, however, the increase of blood pressure is prevented by a simultaneous withdrawal of blood from the animal, no diuresis is observed.

These apparently convincing results were contradicted by Magnus, who found that a solution of sodium sulphate produced twice as great diuresis as an equal volume of an isosmotic solution of sodium chloride, and the increase in volume of the kidney was not twice as great in the case of the former as in the case of the latter, but was, on the contrary, less.

Conflicting as much of the evidence is, Hamburger calls attention to the following points, by way of initiating a physical theory of urine secretion.

1. Attention is called to the presence of fine blood vessels surrounding the glomerulus capsules; and since this blood will have a higher osmotic pressure than the liquid in the intracapsular space, formed by filtration, absorption into them will occur. This exercises an attraction on the glomerulus filtrate which assists the filtration pressure, and so it would be possible to get filtration of urine into the capsules with a blood pressure less than 25 mm. of mercury. (It is recognised that the matter is more complicated than this, as probably the permeability of the capsular wall is not the same as the epithelium of the glomeruli, etc.)

2. Since the filtration in the glomeruli does not take place in air but in liquid, the process will be assisted by diffusion, so that the liquid which passes to the tubules is not the result of filtration merely, but also of diffusion; and as the relative importance of these processes alters, so also will the composition of the urine alter.

3. In the tubules, filtration, but chiefly diffusion, takes place, and as the form of the epithelial cells is different at different parts, it is probable that the permeability is altered and specialised.

4. The diminution of urine secretion which accompanies the diminution of flow of venous blood, is attributed to the presence of carbon dioxide, whereby a swelling of the epithelium is produced and the production of urine is restricted.

VELOCITY OF REACTION AND CATALYSIS.

When a chemical reaction occurs, it does not do so instantaneously, but requires time. By studying the velocity with which a reaction takes place, valuable information is often obtained with regard to the mechanism of the reaction, and the quantitative effect of various additions on the velocity can also be studied.

The basis of the study of reaction velocity is furnished by the law of mass action, which has already been employed to define the conditions of equilibrium. This law states that the velocity with which a reaction occurs is proportional to the concentrations of the reacting substances; or since the velocity is measured by the amount of substance changed divided by the time required for the change to occur, we see that the change of concentration of the reacting substances in unit time will be proportional to the concentrations of the reacting substances during that interval of time. Since, however, the concentrations are continually and continuously changing, the velocity which is thus obtained is only a mean velocity during a particular interval. In order to test the law, however, it is necessary to know the velocity at any given moment, that is, we must determine the change of concentration over an interval so short that the velocity during that interval can be regarded as constant. Since this would necessitate dealing with intervals of time immeasurably small, it would appear as if the law could not be tested. With the help of the differential and integral calculus, however, one is enabled to overcome this difficulty, and to obtain an expression by means of which the velocity at any given moment can be calculated, and which nevertheless, involves only measurable quantities. Into the details of this it will be unnecessary to enter here; and it will suffice to say that expressions of different forms are obtained according as the number of interacting molecules is one, two, three, etc.

In illustration of what has been said, we may take two simple cases: When arseniuretted hydrogen is heated, it decomposes into arsenic and hydrogen, according to the equation

$$AsH_3 = As + 3H$$

In this case only one molecular species is undergoing change, and the expression which allows us to follow the course of the reaction at any moment is given by $\frac{1}{t}\log_e \frac{a}{a-x} = k$ where t is the time measured from the beginning of the reaction, a is the concentration of the substance at the commencement, x is the amount changed, and k is a definite number which is characteristic of the reaction at any given constant temperature.

When the concentration of two different molecular species is simultaneously undergoing change, as, for example, in the case of the saponification of an ester with an alkali, as represented by the equation

$$CH_3 \cdot COOC_2H_5 + NaOH = CH_3 \cdot COONa + C_2H_5OH$$

then an expression of the form $\frac{x}{t(a - x)a} = k$ is obtained, supposing that we commence with equivalent quantities of the reacting substances (ethyl acetate and caustic soda). This expression differs from the preceding one, but in this case also a definite number is obtained which is also characteristic of the particular reaction at a given constant temperature.

By determining the value of the characteristic constant k, called the velocity constant of the reaction, in the case of different reactions, we can compare the velocities of different reactions with one another; and not only so, but we can also study the effect of different added substances on the velocity of a particular reaction. That additions of foreign substances very often do markedly affect the velocity of a reaction, without themselves apparently taking any part in the reaction, has been known for long, although it is only within comparatively recent years that the quantitative effect has been much studied, on the basis of the principles outlined above. Substances, such as we have just referred to, which affect the velocity of a reaction—either increase or diminish it—without themselves undergoing any apparent change, are known as catalytic agents, or catalysers.*

One very well-known reaction which can be catalytically accelerated is the inversion of cane sugar. If cane sugar is boiled with water, inversion into glucose and fructose occurs, but with very great slowness. On adding a small quantity of hydrochloric, sulphuric or other acid, however, the process of inversion takes place very much more readily. On investigating the effect of addition of various acids in varying amounts, it was found that the accelerating effect is proportional to the concentration, not of the acid, but of the hydrogen ion formed by the ionisation of the acid. A strong acid will therefore cause a greater acceleration of the inversion than an equivalent amount of a weak acid. In fact, one can employ the method for determining the strength of an acid.

---

* The term catalyser applied to a substance producing catalysis, carries with it, of course, no explanation of how the effect is brought about. The latter is at present being made the subject of not a few investigations which have yielded very probable explanations of how the acceleration of reactions is brought about in many cases. The definition of catalyser has also been extended.

Similarly, it was found that the velocity of saponification of esters depends not on the total concentration of the alkali, but on the concentration of the hydroxyl ion.

The quantitative method of study which has been applied with such success to these reactions, has also been recently employed in the investigation of another class of catalysers, the ferments and enzymes formed from them. Cane sugar, you are aware, is inverted not only by water in presence of acids, but also in presence of the enzyme invertase. Although it has been found that the action of the latter is in some ways similar to that of acids, it is more complicated, and the physico-chemical investigation of the complicating factors is one of great importance. In all cases, however, the method of investigation is the same, and depends on the quantitative study of the velocity of reaction.

One of the most striking and important features exhibited by catalytic phenomena is that the effect produced is often out of all proportion to the amount of the catalyser; and this is a peculiarity which it is of the highest importance for the biologist to bear in mind. It is also necessary to remember that the effect produced is not always one of acceleration, but sometimes also of retardation. Moreover, the accelerating effect of one catalyser may be often compensated or more than compensated by the addition of perhaps an infinitesimally small amount of some other substance. Thus, certain substances act as poisons; they act as negative catalysers, or retarders of reaction.

Some light may be thrown on the action of poisons on the organism, by the consideration of some analogies in the inorganic world. Thus, when finely divided platinum is brought in contact with hydrogen peroxide, the decomposition of the latter into water and oxygen is greatly accelerated; and the acceleration is quite appreciable even when the amount of platinum present is almost infinitesimally small. Thus, for example, it was found that the presence of one-three-hundred millionth ($\frac{1}{300,000,000}$) of a gram of platinum in a litre, could be detected by its accelerating action on the decomposition of hydrogen peroxide.

But this accelerating action of the colloidal or finely divided platinum can be overcome, the platinum can be "poisoned"; and in many cases substances which are poisons for the animal organism are also "poisons" for the platinum. Thus, for example, sulphuretted hydrogen, but more especially prussic acid, has a very greatly retarding or "poisonous" effect on the platinum. In fact, it was found that the presence of as small a quantity as one-millionth of a gram of prussic acid in a litre, reduced the action of the platinum to one-half.

Other instances of the decided effect of minute traces of certain substances are not difficult to find. Thus we have the poisonous action on certain organisms of water which has been in contact with copper. The cells of spirogyra, it was found, were poisoned by water which had been in contact with copper for four days, although the amount of copper in the water was only one part in one thousand million of water.

A still more striking case of retardation of a reaction is that of copper on the oxidation of solutions of sodium sulphite by oxygen. In this case the presence of as small an amount of copper (in the form of copper ion) as 6.36×10^{-11} (one-sixty-thousand millionth) of a gram, could be quite distinctly detected. Even allowing the water used in the preparation of the solution to remain for three-quarters of a minute in contact with copper, sufficed to produce a marked reduction in the velocity of oxidation of the sulphite.

Now these phenomena have of course not all been explained; but the important lesson to be gathered from them is the enormous importance which excessively minute amounts of substance may have. Thus, the effect of small quantities of enzymes in the blood may have a very important influence on vital processes, such as the processes of oxidation, etc. Further, the importance of minute quantities of certain substances also becomes more intelligible, as in the case of the iron in hæmoglobin, or the arsenic in the thyroid gland. In the latter case, it has been found that the thyroid gland contains about 0.17 milligram of arsenic, or about one four hundred millionth of the body weight, and yet, even in this trifling amount it appears to be essential to health. Although the purpose of the arsenic has not been explained, the fact that it is present in very minute amount need be no bar to our ascribing to it a rôle of great importance.

Before leaving the subject of reaction velocities, mention should be made of the very great influence of the temperature on the velocity. Although the exact influence of temperature differs in the case of different reactions, it can be stated that as a general rule, an elevation of the temperature by about 10° doubles the velocity of a reaction. Thus, a reaction which requires at 0°C. a period of twenty-four hours for its completion, will at 100° require only about 84 seconds; while at a temperature of –100°, it would require nearly a year and a half.

COLLOIDAL SOLUTIONS.

The study of colloidal solutions is one to which a large amount of attention has recently been devoted; and although many facts have been accumulated and a number of uniformities in the behaviour of colloids have been observed, there are still many points requiring explanation. We shall therefore limit ourselves here to a few notes on certain points in the behaviour of colloids which may have a more or less important bearing on the investigation of physiological problems.

With regard to the nature of colloidal solutions there has been much discussion as to whether they are true solutions or merely suspensions of fine particles. Some of these so-called solutions are undoubtedly suspensions (*e.g.*, arsenious sulphide), for examination under a microscope reveals the presence of particles. In other cases, however, no particles have been observed even with the aid of the microscope; and even examination by means of the recently invented instrument, the ultra-microscope, which reveals the presence of particles of the order 4-6 $\mu\mu$. in diameter (molecular dimensions are 0.1-0.6 $\mu\mu$.), did not result in the discovery of suspended particles (*e.g.*, molybdenum blue).

The existence of an osmotic pressure has been adduced as evidence in favour of colloidal solutions being true solutions; and from the value of the osmotic pressure found, the value of the molecular weight has been calculated. Although the comparatively uniform values thus obtained may be allowed as evidence of the existence of true solutions, it does not necessarily prove it. It does not seem at all improbable that the surface forces (surface tension) existing between the particles of the suspension and the liquid would produce an effect similar to that of osmotic pressure; that is, a certain amount of work would be required to separate the suspension from the solvent. The heat which is evolved on moistening fine powders with water is in support of this.

Apart from this, however, it may quite well be that in the case, say, of starch, which gives an apparent osmotic pressure leading to the value of 25,000 for the molecular weight, a small portion of the starch does exist in true solution, while the greater portion exists as suspension.

Certain other properties of colloidal solutions, also, point to their being suspensions. Thus, incident light is reflected polarised; and colloids move under the action of electric forces, some to the cathode and some to the anode (phenomenon of cataphoresis).

For the study of the properties of colloidal solutions, however, it is of little moment whether they are suspensions or true solutions, with very large molecular weight; for the behaviour of fine suspensions has been shown to be in many ways similar to that of colloidal solutions in which no particles could be observed.

As criteria of colloidal solution, we may take (1) non-diffusibility through parchment, (2) sensitiveness to electrolytes, (3) movement in an electric field.

With regard to the first of these properties, it is one which was noticed half a century ago by Graham, who was the first to study the subject. With regard to the third property, it has been found that colloids when placed in an electric field move, some to the cathode and some to the anode. In this respect they behave as electrically charged particles. Thus ferric hydroxide is a positive colloid, and moves therefore to the cathode; silicic acid is a negative colloid, and moves therefore to the anode; egg albumin acts as a negative or as a positive colloid according as the solution is acid or alkaline. In alkaline solutions it is a negative colloid, in acid solutions it is a positive colloid.

With regard to the action of electrolytes, it has been found that these cause the precipitation of colloidally dissolved substances, and the degree in which this property is manifested appears in many cases to depend on the electrical charge of the ion, rather than on the chemical nature of it. Thus, in the precipitation of negative colloids, *e.g.*, silicic acid, it is the positive charge on the ion that is of importance. This is apparent from the following table :—

Silicic acid is coagulated

immediately by	in 10 minutes by	in 24 hours by
$Al_2(SO_4)_3$ $[Al^{\cdot\cdot\cdot}]$	$CuSO_4$ $[Cu^{\cdot\cdot}]$	K_2SO_4 $[K^{\cdot}]$
	$CuCl_2$ $[Cu^{\cdot\cdot}]$	$NaSO_4$ $[Na^{\cdot}]$
	$Cd(NO_3)_2$ $[Cd^{\cdot\cdot}]$	
	$BaCl_2$ $[Ba^{\cdot\cdot}]$	

The higher the positive charge, the more readily does precipitation take place.

In the case of a positive colloid, however, it is the negative ion which is effective. Thus,

Ferric hydroxide is

coagulated immediately by	not coagulated by
$Al_2(SO_4)_3$ $[SO_4'']$	$CuCl_2$ $[Cl']$
$CuSO_4$ $[SO_4'']$	$Cd(NO_3)_2$ $[NO_3']$
K_2SO_4 $[SO_4'']$	$BaCl_2$ $[Cl']$
Na_2SO_4 $[SO_4'']$	$Na\,Cl$ $[Cl']$

Further, positive egg albumin will be precipitated by hydroxyl ion : negative albumin by hydrogen ion.

Not only can electrolytes cause the precipitation of colloids, but one colloid can also cause the precipitation of another colloid. In this case, there is a certain optimum concentration of the colloid; too small, or too large an addition, not only does not cause a precipitation of the first colloid, but even exercises a protective influence, rendering the colloidal solution more stable. When one colloid precipitates another, the precipitate consists of both colloids, due to the adsorption of one colloid by the other. This process of adsorption, which resembles but appears to be distinct from solution, is probably due to the action of surface forces, and is therefore all the greater the larger the surface (cf., the removal of colouring matters from solution by means of charcoal). In some cases, of course, the precipitation may be due to the formation of chemical compounds between the colloids; but this does not appear to be a general explanation.

Of the different properties exhibited by colloids, two may perhaps be emphasised as being of especial importance for biologists, viz. (1) the adsorption and precipitation of colloids by crystalloids and colloids; (2) the adsorption of gases.

To illustrate the former, we may take the case of the adsorption of arsenious acid by ferric hydroxide (which is used as an antidote in arsenical poisoning). When solutions of arsenious oxide are shaken up with colloidal ferric hydroxide, so much of the arsenic is taken up by the ferric hydroxide and so much remains in solution; and for different concentrations, it has been found that the relative concentrations of arsenious acid in the ferric hydroxide and in the water is given by the equation $\frac{C_1{}^5}{C_2}=0{\cdot}631$, where C_1 denotes the concentration in the ferric hydroxide, and C_2 the concentration in the water. This expression shows that relatively more arsenious oxide is taken up by the ferric hydroxide from dilute than from concentrated solutions, so that when ferric hydroxide is added to dilute solutions of arsenic, practically all the arsenic is adsorbed by the hydroxide.

As regards the adsorption of gases by colloids, it has been found (by Emslander and Freundlich) that the presence of colloidal substances such as dextrine and albuminoids in beer, enable a much larger amount of carbon dioxide to be taken up by the beer than by a corresponding mixture of alcohol. A similar explanation may possibly hold for the increased amount of carbon dioxide absorbed by blood as compared with a corresponding salt solution.

PHYSICAL CHEMISTRY AND SERUM THERAPY.

Recently some aspects of the problem of immunity have also been studied from the physico-chemical standpoint, but although some interesting analogies have been drawn, it cannot be said that any decisive conclusion has been reached. Time will allow of only an indication of these investigations.

One of the first attempts was made by Arrhenius. As you are aware, the theory of Ehrlich regards the action of an antitoxin on a toxin as due to chemical combination. A difficulty then arose in connection with the fact that the diminution of the toxic action by successive additions of equal quantities of the antitoxin, is not equal, but gradually diminishes (Ehrlich's phenomenon). This cannot be explained on the assumption of complete combination between toxin and antitoxin, and Ehrlich sought to overcome this difficulty by assuming that the toxin consists of a number of components of different toxicity.

From the physico-chemical standpoint, another assumption can be made, viz. : that the reaction between toxin and antitoxin is not complete, but that an equilibrium is established as in the case, say, of acetic acid and alcohol, to which reference was made in a previous lecture. In such a case, the first addition of antitoxin would neutralise a relatively greater amount of toxin than a second equal addition would do, and so on. The action of an antitoxin would then be calculable for any given concentration according to the law of mass action. Experiments by Madsen appeared to support this view in the case of tetanolysin and hæmolysin. In the case of the diphtheria toxin, however, the first addition of the antitoxin does not produce a maximum effect, but practically no effect. This was explained by Ehrlich as due to the presence of a non-toxic substance, a prototoxoid, which combined with the antitoxin. Arrhenius, however, concluded from the experiments of Madsen, that there is no prototoxoid present, nor any so-called epitoxoid and toxones. In this, however, Arrhenius appears to be in error according to the investigations of bacteriologists; and it is better to recognise that the action of diphtheria antitoxin is more complicated than would appear to be the case according to the scheme of Arrhenius. It is, moreover, not beyond question that the application of the law of mass action to the cases in consideration is altogether justifiable.

Somewhat more promising are the experiments by Biltz on the adsorption of colloids, to which reference has already been made (adsorption of arsenious acid by ferric hydroxide). This chemist seeks to explain the action of an antitoxin on a toxin as being due to adsorption of one colloid by another colloid, and in support of this view, various analogies in the behaviour can be cited. Thus, just as there are specific antitoxins for specific toxins, so one colloid cannot be precipitated by all other colloids, but only by certain other colloids. Further, as we have seen in the case of ferric hydroxide and arsenious acid, relatively more adsorption takes place in dilute solutions than in concentrated; and this is also the case in the agglutination of bacteria. When only a small concentration of agglutinin is added, it is practically all taken up by the bacteria; and as the concentration of agglutinin is increased, relatively less and less is taken up, just as in the adsorption of arsenious oxide by ferric hydroxide.

Still another analogy may be mentioned. When an antitoxin is added to a toxin in just sufficient amount to produce a non-toxic solution, the amount of toxin which must then be added in order to constitute a fatal dose is greater than if no antitoxin had been added. This also is found to be the case with ferric hydroxide and arsenious acid. After just sufficient ferric hydroxide has been added to a solution of arsenious acid to produce a non-toxic solution, the further amount of arsenious acid which must then be added in order to act fatally is greater than if no ferric hydroxide had been added.

With this brief indication of a new direction which has quite recently been opened up for attacking the problem of immunity, I propose to conclude this short course. In the short time which has been at our disposal, we have covered a considerable range of study, for the object of these lectures was not to give a full and detailed account of any one point, but rather to afford a glimpse at the large and promising territory which has been opened up by physical chemistry, and to lightly trace some of the directions along which investigators in various departments of biological science, using the methods and guided by the theories and principles of physical chemistry, have penetrated more fully into the nature of their special problems. Considering the very brief period during which physical chemical methods have found application to the study of biological problems, the advance which has been made is very remarkable; and there is every reason to believe that in the future, as the methods become more and more extensively applied, the advance will become more rapid and widespread. This work must, however, be done by the biologist, not by the physicist or chemist; by the biologist who has made himself familiar with the principles and methods of physical chemistry. If these lectures should in any way contribute towards this end, they will have completely succeeded in their object.

LIBRARY
OF THE
UNIVERSITY
OF
CALIFORNIA

THIS BOOK IS DUE ON THE LAST DATE STAMPED BELOW

AN INITIAL FINE OF 25 CENTS

WILL BE ASSESSED FOR FAILURE TO RETURN THIS BOOK ON THE DATE DUE. THE PENALTY WILL INCREASE TO 50 CENTS ON THE FOURTH DAY AND TO $1.00 ON THE SEVENTH DAY OVERDUE.

Mr 27 '3

DEC 20 1937

DEC 9 1939

NOV 9 [illegible]

30Mar'50MW

LD 21-100m-8,'34

YC 21579

145165

QL453
F5

UNIVERSITY OF CALIFORNIA LIBRARY